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SPERMATOGENESIS OF THE BLACK-CLAWED CRAB, *LOPHOPANOPEUS BELLUS* (STIMPSON) RATHBUN.

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INTRODUCTION.

During the past few years I have been studying various cytological aspects of the gonads of numerous Pacific coast Brachyura (Fasten, '17, '18, '21 and '24) giving particular attention to the problem of spermatogenesis. The present paper is a continuation of the above research, dealing with the maturation of the male gametes of the black-clawed crab, *Lophopanopeus bellus* (Stimpson) Rathbun. This Brachyuran is a native of the Pacific coast of the United States, and according to Schmitt ('21), it ranges from Alaska to southern California. The material upon which the present contribution is based was obtained mainly along the shores of Puget Sound. In certain localities of this region, particularly in the San Juan islands, the black-clawed crab is found to be quite abundant.

MATERIAL AND METHODS.

The material used consisted of smear preparations of the testis of the crustacean under consideration. Although sectioned material was available, nevertheless the smears were found to be so superior that all of the stages and drawings were derived from them. The time for obtaining the best material was the latter part of June and the first portion of July. These periods agree quite well with what has been found to be the case in the testes of other Brachyura.

The smears were prepared in the usual manner, as described in my earlier paper on the spermatogenesis of the edible crab, *Cancer magister* Dana (Fasten, '18). Numerous fixatives were tried, but Flemming's strong solution was found to yield the best results. This mixture made the principal parts of the cell stand out clearly. Furthermore, it produced some differentiation in the staining capacities of the chromatoid bodies and the chromosomes. The former invariably stained less heavily than the latter. This was particularly noticeable when the slides were considerably destained. As for staining, the best results were obtained with Heidenhain's iron-alum hæmatoxylin and a counter-stain of acid-fuchsin.

The mature radial spermatozoa were studied in various isotonic, hypotonic and hypertonic solutions in order to discover the manner in which they function. These experiments have already been published in a separate paper (Fasten, 1921) and therefore the results will not be repeated here.

Description of Male Gonads.

The male reproductive organs of *Lophopanopeus bellus* lie in the cephalothoracic region. They consist of a bilobed tubular testis and a pair of vas deferent ducts. Each lobe of the testis runs laterally in the space between the heart and the digestive glands. Directly below the anterior portion of the heart the testicular lobes unite and from this junction point the convoluted vas deferent ducts originate. These run posteriorly to the base of the fifth pair of walking legs where they open to the outside.

The male gonads attain their maximum size during the latter part of June and the early portion of July. Between these times the cells in the tubules of the testis are undergoing rapid proliferation and all stages in the spermatogenesis process may be secured. Typical cross-sections of the testis reveal a similar picture to that given by me in 1918 for the edible crab, *Cancer magister* Dana. Some of the tubules contain just one typical stage; others show two distinct stages, while still others reveal three steps in the maturation process.

In the posterior region of the testis, where the lobes unite, and in the vas deferent ducts are found the mature spermatozoa. In *Lophopanopeus bellus*, the mature spermatozoa within the distal

ends of the vas deferent ducts are surrounded by a single large spermatophore. This is contrary to what has been found in the other Brachyura studied, where numerous spermatophores are developed.

Spermatogenesis.

A. Spermatogonial Stages.

In the black-clawed crab two distinct spermatogonial divisions can be seen (Figs. 1–2 and 8–11). The primary spermatogonial stages (Figs. 1–2) are considerably larger than those of the secondary spermatogonia (Figs. 8–11) but otherwise the division proceeds in the same manner as seen in Figs. 8–11. The resting primary spermatogonium (Fig. 1) is almost twice the size of the resting secondary spermatogonium (Fig. 8). Their structure, however, is similar. Numerous chromatin clumps and linin strands may be distinguished within the nucleus. A large nucleolus may also be recognized. The cytoplasm is uniform throughout, with the exception that occasionally larger masses that stain somewhat like chromatin may be seen. These masses are clearly discernible in Fig. 1. The centrosome which is a single granule is nearly always present.

When any one of the spermatogonia divides, the chromatin within the nucleus begins to fragment until a great many large, heavily staining clumps appear, as shown in Fig. 2. The nuclear wall soon breaks down with the result that the cell enters the metaphase stage of division (Fig. 9). Polar views of the metaphase showed the chromosomes to be rather small and numerous. Accurate counts could not be made although in many cases over one hundred of them were found distributed throughout the equatorial plane. But since it has been definitely determined that there are 62 bivalent chromosomes in the reduced number, there must be 124 univalent ones in the spermatogonial counts.

The anaphase (Fig. 10) and telophase (Fig. 11) stages follow quickly, thereby completing the spermatogonial divisions. The division of the secondary spermatogonia produces the resting primary spermatocytes (Fig. 12) which then continue the maturation process.

In connection with the spermatogonial stages reference must be made to the so-called "nutritive cells" (Figs. 3–7) which are

commonly found associated with the spermatogonial strips located in tubules where there are mature spermatozoa. These so-called "nutritive cells" are huge structures assuming numerous shapes. Typical examples are pictured in Figs. 3–7. The delineation between the cells is oftentimes effaced, so that the nuclei seem to lie in a syncitial mass of cytoplasmic material. There is considerable difference of opinion amongst cytologists as to the probable relation between the nutritive and spermatogonial cells of decapod Crustacea. Grobben ('78), Gilson ('86) and Herrmann ('90) look upon the nutritive cells as foundation germinal cells from which the spermatogonia originate. In contrast to this view, such workers as St. George ('92), and Keppen ('06) regard the nutritive cells as being derived through a transformation of spermatogonia which have not undergone maturation. My own observations are in accord with the latter view. Such stages like Figs. 3 and 4 resemble primary spermatogonial cells (Fig. 1) but are larger in size.

Many of the workers on the cytology of the Decapoda have claimed that the nutritive cells undergo amitotic division. In sectioned material it seems as if this were the case. For instance certain sections through such cells as illustrated in Figs. 5 and 6 would yield typical examples for those advocating amitosis. However, when smears of the entire cells were obtained, the true condition is discovered. I have found numerous indications that mitosis is the normal process of division in these nutritive cells. A good review of the literature on this subject can be obtained in my earlier paper on the spermatogenesis of the American crayfish, *Cambarus virilis* (Fasten, '14).

B. Primary Spermatocyte Stages.

In the primary spermatocyte stages, the cells undergo growth, synapsis and reduction in the number of chromosomes. A single chromatoid body makes its appearance within the cytoplasm during the early stages of the growth period. The general sequence of events is quite similar to that found in other decapod Crustacea (Fasten, '14, '18, '24).

Growth Period.—The resting primary spermatocyte (Fig. 12) is slightly smaller than the resting secondary spermatogonium

(Fig. 8). Within its nucleus may be seen numerous irregular chromatin clumps distributed along a meshwork of thin strands. A single centrosome can also be distinguished.

When the primary spermatocyte begins its process of development, the first noticeable change occurs in the nucleus. The chromatin clumps fragment (Figs. 13 and 14) and soon weave out into a large number of leptotene threads (Fig. 15). These threads are distinct but so numerous that it was impossible to get any count of them.

The next step in the growth process is the pre-synaptic stage (Fig. 16) in which the leptotene threads wander to one pole of the nucleus and become lined up side by side into pairs. Within the cytoplasm a single, spherical chromatoid body makes its appearance and it is invariably surrounded by a clear area. This is quite similar to the bodies found by Wilson ('13), Komai ('20) and myself ('14, '18 and '24) for other forms. In slides which were considerably destained the chromatoid body took on a lighter hue than the chromatin of the nucleus. I found this same differentiation in destained preparations of *Cancer productus* Randall, *Cancer oregonensis* (Dana) Rathbun, and *Cancer gracilis* Dana which were fixed with Flemming's strong solution. From this stage on the chromatoid body persists and can be easily distinguished.

The pachytene stage follows (Fig. 17) in which synapsis of the paired leptotene threads takes place thereby transforming them into distinct gemini. In many of the preparations the line of fusion between some of the leptotene threads could be clearly seen. This is similar to the condition found in *Cambarus virilis* (Fasten '14), but differs from that found in the various Cancer crabs studied (Fasten, '18 and '24) where the fusion between the leptotene threads is complete.

The diplotene and post-diplotene stages (Figs. 18 and 19) follow each other in logical sequence. In the diplotene stage the paired threads of each geminus open up at one end along the longitudinal plane of fusion but remain attached at the opposite end, thereby producing figures like V, 8 and U. At the same time a second longitudinal furrow occurs along each of the opened arms, thus converting each geminus into four thin strands

attached at one end, as seen in some of the V's, U's and 8's of Fig. 18.

The four components of each geminus continue to open up in such a manner as to produce typical X's like those shown in Fig. 19. This transformation marks the end of the post-diplotene stage and the cell is now ready to terminate the growth period by the formation of tetrads.

Figure 20 shows the pre-tetrad condition. The opposite ends of the X's soon wander towards each other and the middle fusion point becomes effaced. The result is that every one of the X's is converted into four thin strands, having the appearance of a pair of parallel lines with a transverse split in the middle.

Through a further condensation, each of the four thin strands is modified into a spherical chromosome, thus converting every geminus into a tetrad (Figs. 20 and 21). Following this, the typical bivalent dumb-bells are formed through a fusion of the pairs of univalent chromosomes of the tetrads. This is particularly well shown in Fig. 21. Here, also, the centrosome has divided into two elements and each is seen to migrate away from the other. At the same time the nuclear wall has begun to disintegrate and the cell, in general, has terminated its process of growth.

A careful study of the various stages of growth shows that the chromatoid body makes its appearance in the pre-synapsis period and persists from then on. A centrosome and an idiozome are clearly visible. As for synapsis, it occurs in parasynaptic fashion in which there is a side-by-side conjugation of the chromosomes. This is similar to what happens in the other decapod Crustacea which I have studied (Fasten, '14, '18 and '24). Komai ('20) has also found parasynapsis in the crustacean *Squilla oratoria* de Haan.

Reduction Division.—Following the disintegration of the nuclear wall, the primary spermatocyte enters the metaphase period (Figs. 22 and 23). The bivalent dumb-bells are lined up in the equatorial plane, and from them the delicate spindle fibres converge towards the centrosomes which are located at opposite poles. The single chromatoid body wanders undivided towards one of the poles of the cell. In some instances it lies amongst

the spindle fibres (Fig. 22) while at other times it is located outside the spindle proper, as shown in Fig. 23. As already mentioned, when the slides were greatly destained, the chromatoid body could be easily distinguished from the chromosomes as it took on a lighter color. The most consistent counts of polar views of metaphase stages showed 62 bivalent chromosomes distributed throughout the plane of the equator (Fig. 24).

The anaphase and telophase stages (Figs. 25–28) follow quickly, ultimately producing the secondary spermatocytes. Figure 28 shows the two types of secondary spermatocytes formed, one containing the single chromatoid body, while the other is devoid of any such structure. These two kinds of secondary spermatocytes occur in equal number.

C. Secondary Spermatocyte Stages.

The secondary spermatocyte divisions are equational. No rest period is found after the mitosis of the primary spermatocyte, the cells immediately enter the metaphase to undergo rapid division. No good counts of the chromosomes of secondary spermatocytes could be made. The cells on the whole are almost half the size of the primary spermatocytes.

Figures 29–34 show the typical stages in the division of the secondary spermatocyte which possesses a chromatoid body, while Figs. 35–38 show the same process in the secondary spermatocyte that is minus the chromatoid body. The ultimate result of all these divisions is the formation of two kinds of spermatids, one which contains a chromatoid body (Fig. 39) and the other that lacks it (Fig. 44). This latter spermatid is about three times as numerous as the former one.

D. Transformations of Spermatids.

The two types of resting spermatids (Figs. 39 and 44) are spherical cells which have large masses of chromatin within the nuclei that stain intensely black with Heidenhain's hæmatoxylin. The cytoplasm is uniformly granular, possessing a distinct centrosome. The chromatoid body can be clearly seen within one of the kinds of spermatids (Fig. 39).

Transformations of the two spermatids occur in similar fashion.

The first change is a disintegration of the dense chromatin of the nucleus. This is consistently reduced until there are two spherical granules (Fig. 45) and then one (Figs. 40 and 41) left. These single granules appear like karyosomes. While all this is going on the nucleus takes on a lighter stain and at the same time, a mass of dense material makes its appearance in the cytoplasm which stains like chromatin (Figs. 40, 41 and 45). Koltzoff ('06) and Binford ('13) regard this material as mitochondria. I think this is nuclear material which has diffused out into the cytoplasm as the chromatin content has become reduced. I found a similar condition of affairs in the spermatids of *Cancer magister* (Fasten, '18).

Soon a vacuole makes its appearance at one end of the cytoplasm, the nucleus wanders towards the opposite pole and the centrosome with the mitochondria-like mass take the middle position between them (Figs. 42, 43, 46 and 47). In the spermatid which contains the chromatoid body, all changes go on as usual, but the chromatoid body wanders to the periphery of the cytoplasm and is ultimately eliminated from the cell (Figs. 39–43), thus playing no further part in the transformations. From now on the spermatids seem to be similar and the same changes take place in them.

The mitochondrial mass soon becomes ring shaped and the centrosome occupies its center. The upper end of the nucleus has penetrated the open inner space of the mitochondrial ring as seen in Figs. 48 and 49. The karyosome-like granule has also wandered upward until it comes to lie directly below the centrosome. The spermatid now appears like Fig. 49.

Subsequent to this stage there is a fusion between the centrosome and the karyosome-like body of the nucleus to form a single structure. This then elongates to look like a short rod (Fig. 50). Going hand in hand with these changes, a bubble makes its appearance at the upper end of the first vacuole (Fig. 50), which gradually increases in size (Figs. 50–55) and becomes the second vacuole. In *Lophopanopeus bellus* a careful study of the smear preparations reveals the fact that this second vacuole was formed through a diffusion of substance from the distal end of the central rod. This is clearly shown in the figures

indicated. The second vacuole increases in size and ultimately surrounds completely the central body so that the spermatid presents the picture illustrated by Fig. 55. In this figure the primary and secondary vacuoles have changed into distinct vesicles and from now on they will be designated as the primary and secondary vesicles.

Soon a thin line grows out from the distal portion of the central body and this penetrates the upper end of the second vesicle to form a small opening (Fig. 56). The central body then begins to hollow out thereby becoming tubular. At the same time the outer opening of the second vesicle becomes collar-like in appearance. These changes are seen in Figs. 57–59. Following this the nucleus and mitochondrial ring fuse and become like one structure drawn up tightly around the vesicles (Fig. 60). From this last named nuclear-mitochondrial portion, the radial arms of the mature spermatozoa are formed (Fig. 61).

The last transformation in the mature spermatozoa occurs in the central body. The proximal end of this structure penetrates the tubular, distal end to form a small spine-like vertical rod (Figs. 62 and 63). This can only be seen in slides of spermatozoa which have been greatly destained. In other slides the spermatozoa all present the appearance of the one shown in Fig. 64. When such a male gamete that has been stained with Heidenhain's hæmatoxylin and acid-fuchsin is examined, the central body is uniformly black except for its distal end where it joins the dark collar-like opening, which is clear; the second vesicle is an amber color; the primary vesicle is colorless, and the nuclear-mitochondrial mass is darkly granular, with a darker ring immediately around the second vesicle. Such mature spermatozoa fill in the lower portion of the testis and the vas deferent ducts.

When these spermatozoa begin to open up one notices that four types of them exist, a three (Fig. 65), four (Fig. 66), five (Fig. 67) and six (Fig. 68) rayed type. The four and five rayed types predominate in largest numbers. The opening up and explosion of these spermatozoa has already been described in a previous contribution to this journal (Fasten, '21). As pointed out in that paper, the explosion is produced by a lowering in the osmotic pressure of the medium which surrounds the gametes.

Summary.

1. During the latter part of June and early portion of July the testis of *Lophopanopeus bellus* (Stimpson) Rathbun is in the best condition for the study of the spermatogenesis process.

2. Primary and secondary spermatogonial divisions can be distinguished. The spermatogonial chromosomes are univalent and probably number around 124.

3. Large nutritive cells are frequently associated with spermatogonial strips in tubules where there are mature spermatozoa. These have irregular nuclei, and are, undoubtedly, produced from primary spermatogonia which have failed to mature.

4. The primary spermatocyte undergoes growth, parasynapsis, tetrad-formation and reduction division. There are 62 bivalent chromosomes seen in polar views of the metaphase stages of this division.

5. During the growth period a chromatoid body appears in the cytoplasm, and when the reduction division takes place this wanders undivided to one of the poles of the cell, resulting in the formation of two kinds of secondary spermatocytes, one which possesses the structure and the other which is devoid of it.

6. There is no rest period between the primary and secondary spermatocytes. The division of the latter is equational and produces two types of spermatids, one having a chromatoid body and the other which is minus such a structure. This last type is about three times as numerous as the former one.

7. At an early stage, the chromatoid body is expelled from the spermatids which contain it, and from then on all the spermatids undergo similar complicated transformations. These changes bring about the formation of the radial spermatozoa which are packed away in single spermatophores within the vas deferent ducts.

8. Four kinds of mature spermatozoa may be distinguished, namely, three-, four-, five- and six-rayed types. The four- and five-rayed spermatozoa are the ones which are encountered most frequently.

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EXPLANATION OF PLATES.

The figures in the accompanying plates were drawn with the aid of the camera-lucida at a magnification of 1,600 times. They are all from smear preparations.

Explanation of Plate I.

Fig. 1. Resting primary spermatogonium.

Fig. 2. Fragmentation of chromatin into smaller clumps, primary spermatogonium.

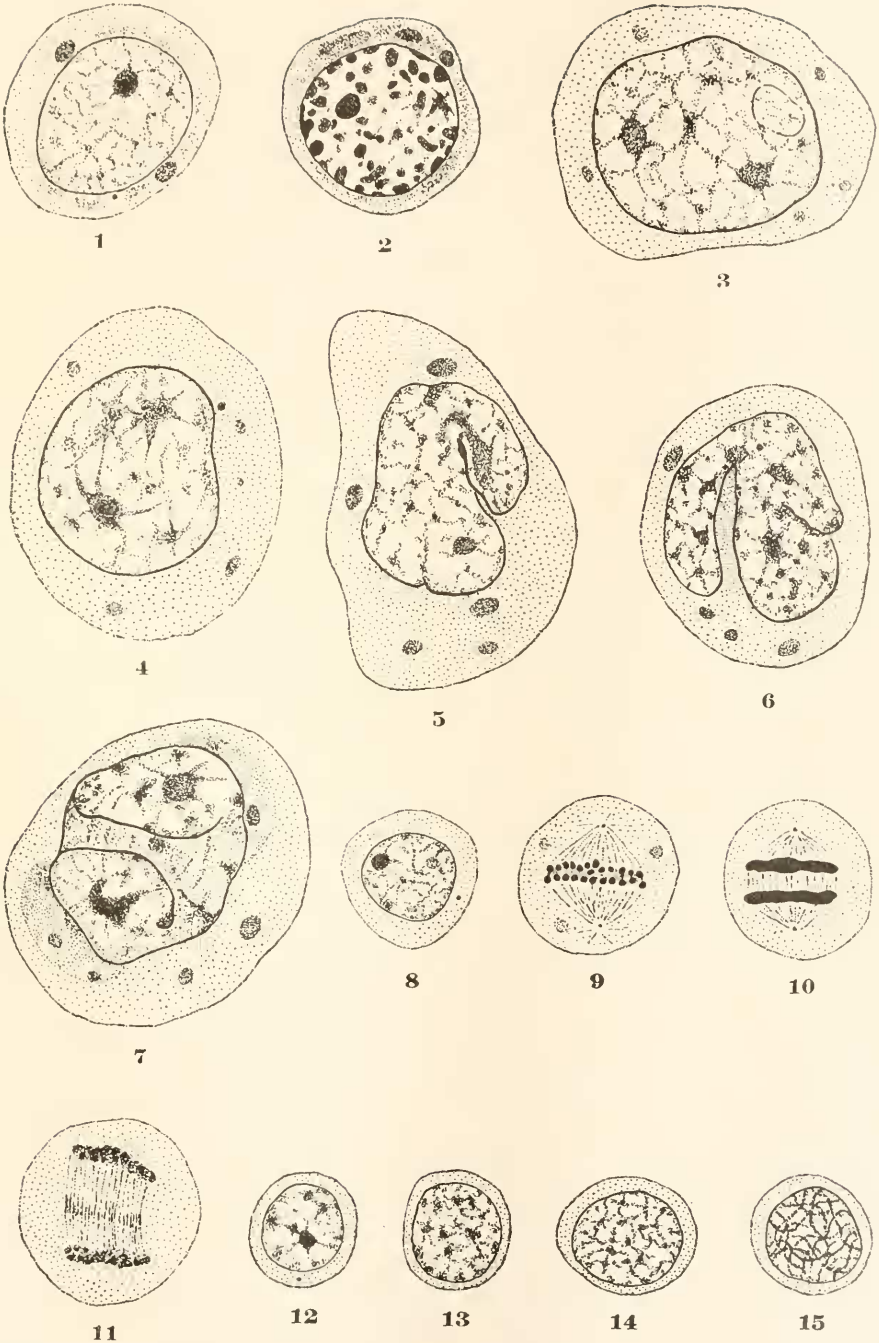
Figs. 3 to 7. Nutritive cells.

Figs. 8 to 11. Secondary spermatogonia in various stages of mitosis.

Fig. 12. Resting primary spermatocyte.

Figs. 13 and 14. Early growth stages, primary spermatocyte.

Fig. 15. Leptotene stage, primary spermatocyte.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Explanation of Plate II.

Fig. 16. Pre-synapsis stage, primary spermatocyte, showing the parallel, side-by-side arrangement of pairs of leptotene threads.

Fig. 17. Pachytene stage, primary spermatocyte.

Figs. 18 and 19. Diplotene and post-diplotene stages, primary spermatocyte.

Figs. 20 and 21. Pre-tetrad, tetrad and dumb-bell formation, primary spermatocyte.

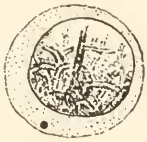
Figs. 22 and 23. Metaphase stages, primary spermatocyte.

Fig. 24. Polar view, metaphase, primary spermatocyte, showing sixty-two bivalent chromosomes.

Figs. 25 to 28. Stages in division of primary spermatocyte.

Figs. 29 to 34. Stages in division of secondary spermatocyte that possesses a chromatoid body.

Figs. 35 to 38. Stages in mitosis of secondary spermatocyte which lacks the chromatoid body.



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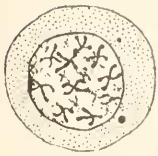

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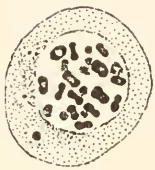

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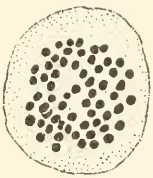

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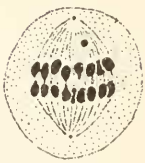

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Explanation of Plate III.

Figs. 39 to 43. Stages in transformation of the spermatid that contains a chromatoid body. In Fig. 43 the chromatoid body is expelled.

Figs. 44 to 47. Stages in the transformation of the spermatid that does not possess a chromatoid body.

Figs. 48 to 64. Later stages in transformation of both types of spermatids.

Figs. 65 to 68. The four types of spermatozoa produced.

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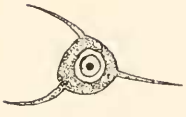

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SOLE PATTERNS OF TWINS.

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In the study of twins, one frequently desires to know whether or not they are of monozygotic or dizygotic origin. This question can be answered by examination of the placenta and membranes at birth, but when older children and adults are studied it is difficult, and often impossible, to do so. Work has been done on the general physical appearance, mentality, palm patterns, whorls of the hair, etc., but no definite data has been secured. This study was undertaken in the hope that the friction-ridge patterns on the sole of the foot might reveal some clue as to the type of twin from whom they were secured.

Newman [1, 2] thinks that monozygotic twins are apt to have identical patterns, that they are found nearly always in this type of twin, but that unlike patterns do not disprove their monozygotic origin. Wilder [3] also has remarked upon the striking similarity which sometimes occurs. He [4] states that while they are similar grossly, the Galton details are always different. Accurate information on this subject is lacking.

Prints were secured from 87 pairs of twins living in Madison and vicinity; the process perfected by Professor Mathews being used in order that the fine details might be more readily studied. The youngest pair was one week old, and the oldest 85 years. Most of the subjects, however, were school children. Thirty-three pairs were male-male, 24 were female-female, and 30 were male-female. Of the 174 individuals examined, 96 were male and 78 female.

The ratio of monozygotic to dizygotic twins has been variously stated. Newman [1] thinks that the monozygotic type occurs about 25 per cent. of the time, while De Lee [5] quotes Ahlfeld as stating that 15.55 per cent. of all twins are monozygotic. Ahlfeld's figures are, perhaps, more reliable because he examined the placentas and membranes in 1,157 twin births.

The Wilder method is used in classifying the patterns and

a summary of his method is as follows: The pattern bearing area on the ball of the foot is divided into two parts, one proximal to the great toe (hallucal) and the other proximal to the smaller toes (plantar). When three ridges meet to form a "Y" it is called a delta of which there are three in the hallucal area, one distal (just below the great toe), one medial, and one lateral. If all three of them are present the pattern is a whorl (W), if the distal one is absent the resulting pattern is a loop opening toward the great toe and is designated as an "A" pattern, if the medial one is absent and the loop opens medially a "B" pattern is formed, and if the lateral delta is not present a "C" pattern results. When no pattern is present it is called an open field (O). The plantar area contains three places in which a pattern may be found. These lie in the three interdigital spaces proximal to the four smaller toes. Four general types of patterns are found in these areas: the open field or no pattern, designated by an O, a loop opening distally (U), a loop opening proximally (∩), and a whorl (W). To illustrate the application let us turn to the right foot of twin 8 (Fig. 1). The hallucal pattern is a loop opening distally and is described as an 'A' pattern. The first plantar area also contains a loop opening distally but is designated by a 'U,' as is the second plantar area. In the third area there is no pattern (O). Bringing the various symbols together we have AUUO. To simplify matters, Wilder has proposed a table in which each combination of plantar patterns is given a number: O O O = 1, O O U = 2, O O ∩ = 3, etc. The combination here is 21, and the formula is thus abbreviated to A21. Proximal to the plantar areas there are frequently found other deltas which are designated by the symbol 'd,' and as there are two in this print, we arrive at the formula A21dd. To formulate the left foot we proceed in the same manner except that the plantar patterns are still read from left to right, giving the formula A6dd. The formula of the right foot is placed as the numerator of a fraction and the left foot as the denominator:

$$\frac{\text{A21dd}}{\text{A6dd}}.$$

In order that a set might be called identical the following conditions must be met: (1) either all four patterns, or both

right and both left patterns must have the same formula, and (2) there must be no marked differences in the patterns even if they have the same formula. As space does not permit a more detailed description of the Wilder method of classification, the reader is referred to his writings on the subject. Fig. 1 illustrates

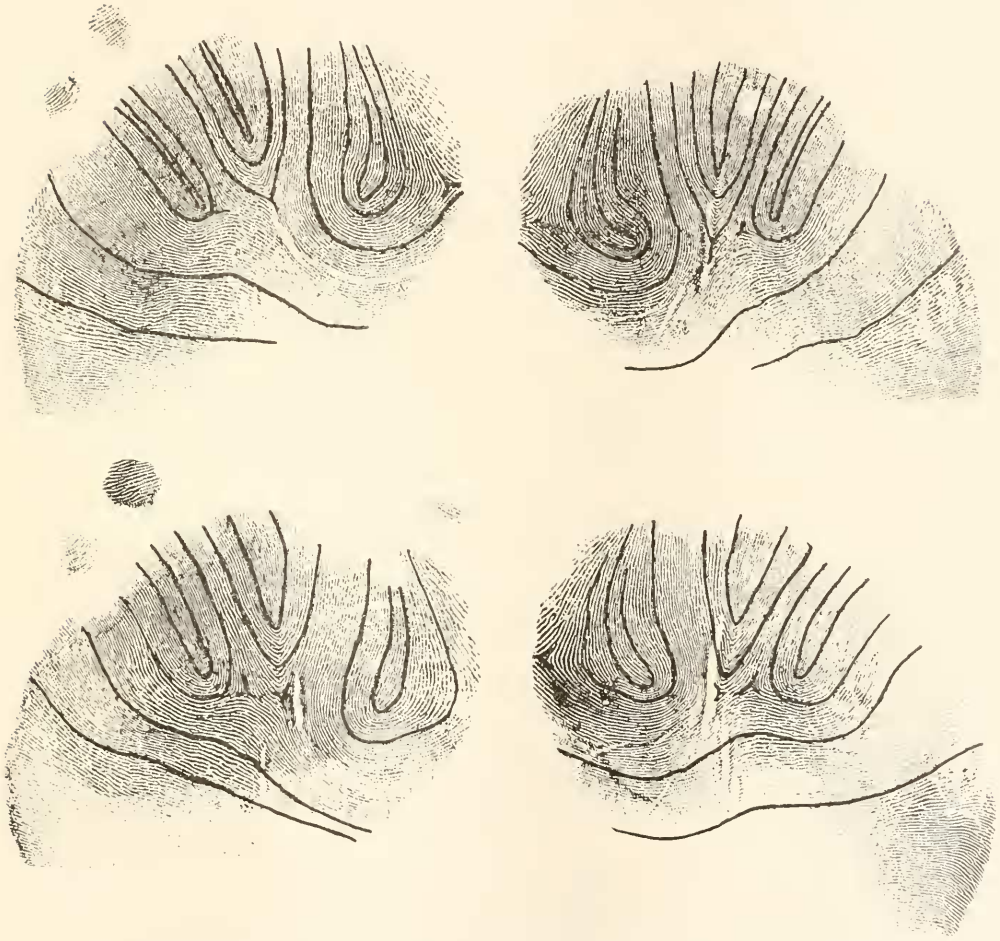

FIG. 1. Twins 7–8. Formula: $\frac{A21dd}{A6dd}$. Some of the lines have been inked in order that the patterns might be more readily seen.

a set of identical patterns. On close examination the similarity of the patterns of each individual is striking. In this series 13 sets (14.94 per cent.) are identical (Table II.), and all except

one occurred in like-sex twins. The striking points of similarity in the different sets are enumerated below. Table I. gives the complete list of patterns. Although twins 25–26 have the same formula, they are not classed as identical because of the very rudimentary character of one of the patterns and so do not meet the conditions stated above.

Table I.

Complete List of All Twin Patterns.[1]

No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.
1	♀	W38dd	W23dd	59	♀	A5d	A5d	117	♀	W5d	B5d
2	♂	A1	A1	60	♀	A5d	A21dd	118	♀	W7d	W1
3	♂	A37d	A5d	61	♂	A5d	A5d	119	♂	A6dd	A21dd
4	♂	A5d	A1	62	♂	A1	A5d	120	♂	A6dd	A21dd
5	♂	W25d	B5d	63	♀	A17d	A1	121	♀	A1	A1
6	♂	B5d	B5d	64	♂	W13d	W13d	122	♂	A1	W3
7	♀	A21dd	A6dd	65	♂	A5d	A1	123	♂	A6dd	A21dd
8	♀	A21dd	A6dd	66	♂	A5d	A5d	124	♂	A6dd	A5d
9	♀	W14ddd	W13d	67	♂	A5d	A5d	125	♂	A5d	A5d
10	♀	W12	W5d	68	♂	A5d	A1	126	♀	A6dd	A1
11	♂	B5d	A5d	69	♂	A1	A1	127	♀	A5d	A11
12	♀	A1	A1	70	♀	A37d	A7d	128	♀	W5d	A5d
13	♀	A29dd	A6dd	71	♀	A37d	A7d	129	♂	O45d	O15
14	♀	A5d	A5d	72	♂	A57d	A1	130	♀	A13d	W15d
15	♂	W5d	W5d	73	♀	A6dd	W5d	131	♂	O1	A1
16	♂	W5d	W5d	74	♀	W5d	W5d	132	♀	A5d	B1
17	♂	A5d	A5d	75	♀	B1	B1	133	♂	A1	A1
18	♂	A37d	A7d	76	♀	B1	A1	134	♀	A1	A1
19	♀	W5d	W5d	77	♀	B45d	W7d	135	♀	W5d	W5d
20	♀	W5d	W5d	78	♀	B45d	W7d	136	♀	A1	A1
21	♀	A6dd	A1	79	♀	W1	W1	137	♀	A1	A1
22	♂	W33	W1	80	♀	A5d	A3	138	♀	B5d	A5d
23	♂	A37d	A5d	81	♀	W6dd	W5d	139	♂	A5d	A7d
24	♂	A5d	A5d	82	♀	A5d	W5d	140	♂	W5d	W5d
25	♂	W5d	W5d	83	♂	W49d	W4d	141	♂	W5d	W5d
26	♀	W5d	W5d	84	♂	W61dd	W12d	142	♀	A2d	A7d
27	♀	A5d	A5d	85	♂	A5d	A5d	143	♀	W1	W3
28	♂	W45d	W15d	86	♂	A5d	A5d	144	♀	A37d	W1
29	♂	A5d	A1	87	♀	A45d	A9	145	♂	B1	B1
30	♂	A5d	A1	88	♂	A45d	W13d	146	♂	A33	A1

TABLE I.—(*Continued.*)

No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.
31	♀	A5d	A5d	89	♀	A5d	A5d	147	♀	A2d	A5d
32	♂	B14dd	B29dd	90	♀	W1	W5d	148	♂	A37d	A5d
33	♂	W5d	W5d	91	♂	A5d	A5d	149	♂	W13d	A43
34	♀	A37d	A3	92	♂	A52dd	A6dd	150	♂	W41d	W15d
35	♂	A1	A1	93	♂	W2d	W21dd	151	♂	A5d	A5d
36	♂	A1	A1	94	♀	B6dd	B21dd	152	♂	A5d	A5d
37	♂	A1	A1	95	♂	A5d	A1	153	♂	A5d	A5d
38	♀	A5d	A1	96	♀	B6dd	B5d	154	♂	A6dd	W5d
39	♀	B38dd	B7d	97	♀	A1	W1	155	♀	O38d	O5
40	♂	W37d	W7d	98	♂	A5d	A5d	156	♀	O46d	A15d
41	♂	A6dd	A21dd	99	♀	A33	W9	157	♀	A17d	B1
42	♂	A5d	A7d	100	♀	W45d	W11	158	♂	W5d	W5d
43	♂	W46dd	W7d	101	♂	A1	W1	159	♀	W1	W1
44	♂	A45d	W15d	102	♂	A5d	W5d	160	♀	A5d	A5d
45	♂	A5d	W1	103	♂	W1	W1	161	♂	A33	A2d
46	♂	A1	A1	104	♂	W1	W1	162	♂	W5d	B5d
47	♂	A2d	A17d	105	♂	W5d	W5d	163	♂	W5d	W5d
48	♂	A5d	A5d	106	♂	B1	B5d	164	♂	B6dd	B23dd
49	♀	W5d	A5d	107	♂	A5d	O5	165	♂	A1	W1
50	♀	A5d	A5d	108	♂	W37d	W7d	166	♂	A5d	A5d
51	♂	A1	A1	109	♀	A5d	A1	167	♀	A38dd	A1
52	♀	A17d	A2d	110	♀	A5d	A1	168	♀	A6dd	A21dd
53	♂	W37d	W47d	111	♀	O15d	O29d	169	♀	A41	A11
54	♀	A5d	A21dd	112	♂	W57d	W3	170	♀	A41	A11
55	♂	A5d	W5d	113	♀	A1	A5d	171	♂	A37d	A3
56	♀	W37d	W7d	114	♀	A1	W1	172	♂	A1	A5d
57	♂	A5d	W7d	115	♂	W13d	W5d	173	♂	A38dd	A63dd
58	♀	W13d	W47d	116	♂	W37d	W7d	174	♀	A5d	A7d

[1] In this table ♂ means male, and ♀ means female. The twins are grouped in pairs.

Twins 7–8.—Note the odd shape of the hallucal pattern "A" in both right feet and the pointed character of the first plantar pattern in all four feet. The plantar patterns of the "U" type are usually rounded. The ridge counts from the cores of the hallucal patterns to the cores of the first plantar patterns are: 7R, 37; 7L, 38; 8R, 38; 8L, 39. From the first to the second plantar patterns the count is: 7R, 28; 7L, 30; 8R, 27; 8L, 29.

Table II.

This Gives Only those Patterns which Are Identical.[2]

No.	Sex.	Right.	Left.	No.	Sex.	Right.	Left.
7	♀	A21dd	A6dd	103	♂	W1	W1
8	♀	A21dd	A6dd	104	♂	W1	W1
15	♂	W5d	W5d	109	♀	A5d	A1
16	♂	W5d	W5d	110	♀	A5d	A1
19	♀	W5d	W5d	119	♂	A6dd	A21dd
20	♀	W5d	W5d	120	♂	A6dd	A21dd
29	♂	A5d	A1	133	♂	A1	A1
30	♂	A5d	A1	134	♀	A1	A1
35	♂	A1	A1	151	♂	A5d	A5d
36	♂	A1	A1	152	♂	A5d	A5d
77	♀	B45d	W7d	169	♀	A41	A11
78	♀	B45d	W7d	170	♀	A41	A11
85	♂	A5d	A5d				
86	♂	A5d	A5d				

Twins 15–16.—Although this is a common pattern, all hallucal patterns are counter-clockwise, and all except one are of the lateral pocket variety.

Twins 19–20.—Here, too, all hallucal patterns are counter-clockwise. The ridge counts from the hallucal to the second plantar patterns are: 19R, 73; 19L, 72; 20R, 83; 20L, 80.

Twins 29–30.—This is a common pattern. The prints are too blurred to do a ridge count accurately.

Twins 35–36.—All show finer lines in the plantar areas although there are no patterns present.

Twins 77–78.—Both right feet and both left feet have the same pattern. The plantar areas are of a type not usually seen.

Twins 85–86.—There are suggestions of patterns in the third plantar areas on all four feet.

Twins 103–104.—All hallucal patterns are of the seam variety.

Twins 109–110.—Both right feet and both left feet have the same pattern. The left feet (and not the right) have fine lines in the plantar areas although there are no patterns present.

[2] In this table ♂ means male, and ♀ means female. The twins are grouped in pairs.

Twins 119–120.—The general type of patterns found are not a very common variety. The ridge counts from the second to the third plantar areas are: 119R, 39; 119L, 37; 120R, 27; 120L, 25.

Twins 133–134.—In this set the twins are of opposite sex. This type of pattern is of common occurrence.

Twins 151–152.—The plantar patterns on both left feet have a "V" shape.

Twins 169–170.—This type of pattern is uncommon.

An attempt was made to count the ridges from the various landmarks, but they vary so greatly in most instances that it was not thought worth while to report all of the figures. The striking similarities are given above.

The individual patterns occur in almost the same ratio as those found in a previous series.[6]

The fact that twins 133–134 are of opposite sex might lead one to conclude that study of the sole prints is without value in determining the type of twin under consideration, but I feel that such is not the case. They are of the A1 type, one of the simplest and commonest. I have found that A1 patterns occur about 10 per cent. of the time on both feet, and so do not think it unreasonable to assume that their presence in this set of twins is an accident.

In order to determine whether or not patterns of the same formula might be found on all four feet of ordinary (not twin) brothers and sisters, the prints of 38 pairs (the only ones available at this time) were examined. Of these 10 were female-female, 12 male-male, and 16 female-male. One pair of sisters had the same patterns, but they were of a very common type, A5d.

Conclusions.

A study of the above data leads one to the conclusion that the presence of identical patterns on the soles of a pair of twins might point to their monozygotic origin, but as Newman states, their absence does not disprove it. A study such as this is, of course, not conclusive, but it points the way for further investigation. It was undertaken in the hope that sole patterns might reveal some information as to the origin of twins. If the

placentas and patterns of a large series could be examined at birth, the question would be answered.

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SPERM FILTRATES AND DIALYZATES.

Their Action on Ova of the Same Species.[1]

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Introduction.

The idea persists that spermatozoa bear a specific substance which is essential in fertilization. Yet all attempts to extract from spermatozoa a substance which will cause development of ova of the same species have failed or have been open to grave criticism. It is now possible to avoid certain errors which invalidated the results of these investigations. The experiments, to be reported here, were undertaken to determine whether, by processes of filtration and dialysis, solutions can be obtained from living sperm which will effect activation and development of ova of the same species. For interesting me in this problem and for invaluable aid in its solution I am indebted to Dr. Otto C. Glaser.

The experimental work was carried on during the summers of 1919–1922 at the Marine Biological Laboratory at Woods Hole, Massachusetts, and from December until June 1920–1921 at the

[1] Thesis submitted in partial fulfillment of the requirements for the degree of Ph.D. at the University of Michigan. Contributions from the Department of Zoölogy, Smith College, No. *138*.

Hopkins Marine Laboratory at Pacific Grove, California. I wish to express my appreciation of the hospitality extended to me at these laboratories, and my thanks to their Directors, Dr. Frank R. Lillie and Dr. Walter K. Fisher, for their aid and encouragement.

The fact that the spermatozoa, rather than the medium which carries them, are the essential agents in fertilization was established in 1824 by Prevost and Dumas; and it is now conceded that fertilization is monospermic and involves the combination of nuclear material from a single spermatozoön and a single ovum. In many species only a single spermatozoön penetrates the cytoplasm of the egg, as was shown by Hertwig (1876); but even when in normal fertilization more than one enters the egg, as in the pigeon, the pro-nucleus of only one enters into the formation of the fertilization nucleus (Harper, 1904, and Blount, 1909). As stated by Glaser (1915), "a single spermatozoön is sufficient to carry on the biparental effect."

It is well known, however, that in the process of fertilization in any species the spermatozoa far exceed the ova in number. This was first noted in frogs by Spallanzani (1785), and verified by Prevost and Dumas (1824). Under such conditions direct contact of every ovum with a single spermatozoön is possible. But mere contact does not ensure fertilization. Even in physiological solutions spermatozoa rapidly lose their fertilizing power, Vernon (1899); Gemmil (1900); Dungay (1913); Lillie (1915) and Cohn (1918). Two explanations have been offered in recent years to account for the excess of spermatozoa. Lillie (1915) implies that in an excess at least one spermatozoön *possessing sufficient fertilizing power* ("sperm receptors") will reach each ovum. On the other hand Glaser (1915) and Cohn (1918) state that the extra spermatozoa may produce changes in an ovum which facilitate the inclusion of a single spermatozoön.

The idea of a superficial effect of sperm on ova is supported by results obtained with *Nereis limbata*, Lillie (1911) and Goodrich (1920); with *Arbacia punctulata*, Lillie (1921); with *Sabellaria alveolata* and *Halosydna gelatinosa*, Labbe (1921, 1922); and with *Asterias*, Chambers (1923). There are indications that a chemical substance or substances are produced by the sperm:

Godlewski (1911); Herlant (1912); Heilbrun (1915); and Lillie (1911, 1915, 1921).

What real evidence have we to support the suggestion, recurrent in one form or another, that the sperm exude a substance which facilitates fertilization? The early attempts of Spallanzani (*loc. cit.*), with filtrates, and of Prevost and Dumas (*loc. cit.*), with filtrates and extracts of spermatozoa, to demonstrate such a substance gave purely negative results. In later work: Pierri (1899); Winkler (1900); Du Bois (1900); Gies (1901); Pizon (1905); Jacoby (1910); Morse (1912); Robertson (1912); Foa (1918)—positive results, where reported, are actually vitiated by errors of technique or interpretation, Loeb (1913, p. 104, and 1916, p. 203); and Lillie (1919, pp. 133, 134).

II. Methods.

In an investigation of this kind so much depends upon the methods employed that it seems desirable to present these in some detail.

All glassware was cleaned with potassium-bichromate-sulphuric acid mixture, subsequently removed by washing in fresh and in sea-water. All solutions were made with analyzed chemicals (Kahlbaum's, Merck's, and Baker's) with water redistilled from glass. Specific gravity was determined in early experiments with a standard hydrometer and later with a Westphal balance. A special set of indicators and standards provided and tested at intervals by Hynson, Westcott and Dunning was used for the colorimetric determination of the hydrogen-ion concentration, Clowes and Smith (1923 and 1924). Every possible precaution was taken to avoid accidental insemination and sea-water controls accompanied every experiment.

Method of Obtaining Ripe Reproductive Cells of Sea-urchins.—One method is as follows: The instruments and hands of the operator are thoroughly washed in tap water and the animals are washed in tap water followed by sea-water. They are opened by cutting through the oral disc with sterile scissors, the disc and the alimentary canal are removed with sterile forceps and the cœlom is flushed with filtered sea-water to remove the body fluids and any intestinal contents. Males and females are

placed on opposite sides of the operator. Each animal, unless shedding freely, is immediately wiped and placed on its aboral surface in a Syracuse watch glass. In this position the reproductive cells exude through the genital pores. In a second method suggested by Dr. Glaser, the spines are rubbed off before the animals are washed and dried. If the reproductive elements are ripe, shedding begins immediately and it is unnecessary to cut the "test." There is no admixture with body fluids or with sea-water and very clean dry reproductive cells are obtained. Only ripe spermatozoa are shed and these are collected in a beaker. Not all the eggs shed are ripe. For this reason it is necessary to fertilize a sample of eggs from each female. After this the "certified" eggs are placed together in a finger bowl with sufficient sea-water to keep the concentration of egg-water below a point at which it will injure the eggs.

Method of Obtaining Ripe Eggs of Nereis limbata.—The method recommended by Lillie (1911) and Just (1915*a*) was employed. Males and females as they were caught were segregated in finger bowls of sea-water and kept cool until all preparations for an experiment were complete. The most satisfactory results are usually obtained with reproductive cells taken from such animals within two hours after collection; although, if necessary, shedding can be prevented for twelve hours or more if the dishes containing the animals are kept on ice. Since either drying the animals on filter paper or washing them in distilled water leads to shedding, each animal was "sterilized" by first transferring it to a finger bowl containing two hundred and fifty milliliters of sea-water. (In this volume of sea-water any sperm adhering to the body of the female will lose their fertilizing power.) Subsequently each animal was placed in a dry Syracuse watch glass in which any excess of moisture was absorbed with strips of filter paper, and there cut transversely with sterilized scissors. The eggs or sperm were forced out by the spasmodic contractions of the body muscles. To avoid accidental insemination the males were opened with a second set of instruments; and all inseminated controls were kept separate from the experiments in which special solutions were being tested. Eggs and sperm were kept covered and cool until used.

Preparation of Filters.—Berkefeld and Mandler diatomaceous filters were used: in preliminary experiments the coarse and medium grade of Berkefeld filters; and in all other work Mandler filters, tested to six to twelve pounds air pressure in the size two and one half by five eighths inches. The latter are used generally in bacteriological work. These were boiled in five per cent. aqueous solutions of sodium bicarbonate, washed and boiled repeatedly in redistilled water until the wash water was neutral in reaction. Finally they were thoroughly washed in filtered sea-water. After this a stream of sea-water was passed through the filters until samples of such water produced no injurious effect on unfertilized ova and no disturbance of fertilization or of development. Such filters were considered "clean." Every filter was subjected to this treatment each time that it was used. The necessity for these precautions was indicated by a variety of experiences. The first sea-water passed through a boiled and washed filter may be sufficiently hypotonic to cause cytolysis of ova within twenty-four hours. Unless all alkali is removed the filtered sea-water may produce activation of ova. The use of acid in the cleaning of filters is prohibitive because the filtrates would then contain traces of heavy metal dissolved from the filter bands. The impurities in question are often too slight for detection by chemical tests, but are only too clearly revealed by their effects upon unfertilized or fertilized ova.

Preparation of Sperm Filtrates.—Five and ten per cent. suspensions of spermatozoa were prepared by adding to a definite volume of dry sperm a measured quantity of sea-water. These were allowed to stand at room temperature (fifteen degrees centigrade at Pacific Grove and twenty-two degrees centigrade at Woods Hole) for from five minutes to four hours. To ensure the greatest activity of the spermatozoa the carbon dioxide generated by them was prevented from accumulating by a thorough aëration of the suspensions. The latter were then either clarified first by centrifuging or by filtering through filter paper, or were transferred directly to a diatomaceous filter. Contamination of the filtrates by back suction was prevented by collecting the filtrates into Pyrex test tubes set in the suction flasks.

During the passage of the filtrate it is necessary to prevent or at least to minimize the destruction of spermatozoa by dehydration or compression. This was attempted by not allowing the surface of the mantle to become exposed to air and by frequent and cautious stirring of the suspension. Some of the spermatozoa are not seriously injured in the process of filtration as indicated by the isoagglutinable and fertilizing power of the sperm remaining on the surface of the mantle at the end of the process.

The filtrates, which are automatically freed from any excess of carbon dioxide in passing through the filter mantle, are then transferred to flasks which are kept tightly plugged and placed in a refrigerator. These solutions remain free from bacteria and retain their peculiar physiological properties for at least a month.

Preparation of Sperm Dialyzates.—There is one objection to the use of sperm filtrates. Some spermatozoa may undergo destruction on the surface of the mantle. To meet this objection I prepared dialyzates of sperm in order to compare their action on ova with that of filtrates.

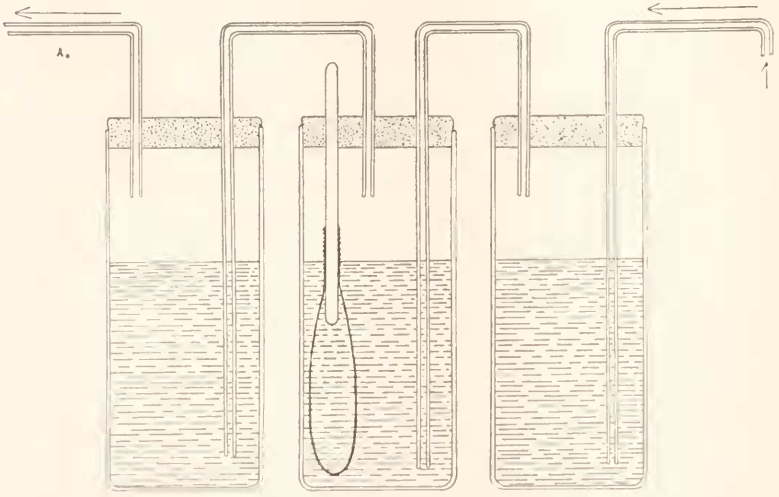


FIG. 1. Dialyzing apparatus. Air is drawn through the sea-water in the three bottles by suction exerted at *A*. The collodion sac is suspended in the sea-water in the central bottle and this sea-water dialyzate used in experiments later. The outer two bottles serve as safety flasks.

Collodion sacs used as dialyzers were made in fifty milliliter centrifuge tubes from a twelve per cent. collodion prepared

according to the method of Gates (1921). The sacs were washed in redistilled water and in sea-water. Samples of sea-water, allowed to stand in the washed sacs for at least twelve hours, were tested in the same manner as that passed through the diatomaceous filters. Tests for leakage were made and any imperfect sacs were discarded. Ten milliliters of twenty-five or of fifty per cent. suspensions of sperm were placed in a tested collodion sac and dialyzed against forty milliliters of sea-water. The resultant dialyzates may be considered comparable to filtrates prepared from five and ten per cent. suspensions of sperm. The sea-water dialyzates must be aërated constantly to provide the most favorable conditions for the sperm during the process. This involves two dangers: evaporation and contamination of the dialyzates. In order to eliminate these sources of error the method illustrated in Fig. 1 was employed.

In these experiments dialysis was continued for five to twenty-four hours. With one exception the sperm in the collodion sacs retained their fertilizing power and their capacity for iso-agglutination at the end of the process.

III. Properties of Preparations.

a. Physical and Chemical Properties.—The osmotic pressure of filtrates and dialyzates, as indicated by the specific gravity tests, is the same as that of sea-water. There is a slight variation in some preparations and in sea-water but it is not sufficient in itself, as determined by experimentation, to produce activation. The hydrogen-ion concentration of filtrates and dialyzates was also equal to that of sea-water (p_H 7.9–8.1 at Woods Hole; 7.6–7.8 at Pacific Grove), indicating that the carbon dioxide formed by active sperm was completely removed by aëration and that the sperm added no other free hydrogen-ions. There is a possibility that small amounts of acid may have combined with buffers in sea-water. The question now arises whether these preparations contain any active physiological principle. No living spermatozoa or fragments were ever observed, nor was there ever a case of normal fertilization in any of the preparations. It is also certain that they contain no chemical substance whose concentration falls within the range of sensitivity of the usual chemical methods.

Experiments in which blood plasma was passed through a Berkefeld filter, Cramer and Pringle (1913), and Goddard (1914), demonstrated that the filter held back for a time, not only suspended elements, but the various proteins in colloidal solution, but the third portion passed through the filter contained some fibrinogen. Mudd (1922) suggested that in alkaline solution protein may be carried through in small amounts. The filter itself is negatively charged and because of the amphoteric properties of protein the latter dissociates as an anion in alkaline solutions and so would tend to be carried through the filter.

It is important therefore to determine if possible whether nitrogen compounds are present since Loeb (1914) states that protamine will induce the first cell divisions in eggs of *Arbacia;* and Labbe (1923) using a one to forty thousand solution of sodium nucleinate in sea-water on unfertilized eggs of *Arbacia* obtained some swimming larvæ. Because of buffer action these solutions had the same hydrogen-ion concentration as sea-water, and Labbe concluded that the sodium nucleinate exerted a specific action not due to its hydrogen-ion concentration.

All the usual tests for protein or protein split products such as guanine, protamine, and nucleic acid were negative. A micro-kjehldahl test, performed for me by Dr. W. Dennis, was also negative, indicating that nitrogen is not present in sufficient amounts to be detected by this method. Incineration tests gave evidence of the presence of carbon in larger amounts in both filtrates and dialyzates than in sea-water.

b. Physiological Properties.—From the presence of carbon in these preparations it is certain that they contain something of organic origin. It seemed possible that they might produce changes in the ova of foreign species, since sperm extracts obtained by other investigators have produced marked effects on ova of foreign species even when they had no influence on ova of the same species, Loeb (1916, p. 102). Consequently tests were made to determine whether *Arbacia* sperm filtrates would affect the ova of *Nereis limbata*, a form in which maturation follows insemination. Seventeen experiments were performed in which ten preparations were used on *Nereis* eggs. The results obtained resemble in many respects those produced by in-

semination with *Nereis* sperm. The foreign filtrate causes production of jelly, the formation of a "fertilization membrane," complete maturation, and segmentation leading to the development of larvæ.[2] The latter however are abnormal in shape and in the distribution of cilia. In no case did these changes occur in sea-water controls. A protocol of one experiment is given below.

TABLE I.

CHANGES IN THE OVA OF *Nereis* EXPOSED FOR TWENTY MINUTES TO A FILTRATE PREPARED FROM A TWO PER CENT. SPERM SUSPENSION OF *Arbacia*.

Date.	Exper.	Per Cent. of Eggs Forming Jelly and Membranes.	Per Cent. of Dividing Eggs.	Per Cent. Swimming.
8/ 4/20	31	100	10	0.5
8/ 5/20	33	99	90	0
8/ 7/20	35	81	75	5
8/ 8/20	37	85	85	9
8/ 8/20	38	100	14.5	2
8/13/20	40	100	1	0
8/14/20	43	100	5	2
8/14/20	44	100	13.5	1
8/14/20	45	100	12	0.5
7/27/21	225	33	31.5	1
7/27/21	225	30	28.5	1

In experiments 31, 40, and 43 the ova were stale. In all cases membrane formation and maturation occurred as rapidly in ova in sperm filtrates as in inseminated ova. Subsequent development in ova treated with filtrate was delayed.

The *Arbacia* sperm filtrate acted like a parthenogenetic agent, yielding results similar to those obtained in *Nereis* with other methods (Lillie, 1911, and Just, 1915).[2] I obtained similar results with filtered egg-water of *Arbacia*. The latter observations, quoted by Dr. Alvalyn Woodward (1921), were verified frequently in subsequent experiments. Further experimentation demonstrated that the effect produced by sperm filtrates on *Nereis* eggs bears a definite relation to the strength of the preparations and to the duration of exposure of ova to them. The main question however is what effect have such filtrates and dialyzates on ova of the same species.

[2] The developing ova divided into two, four and eight cells, and subsequently some of them developed into abnormal ciliated trochophores. The rest cytolyzed.

IV. The Potency of Sperm Filtrates and Dialyzates on Ova of the Same Species.

a. Arbacia punctulata.—The procedure employed to determine the effect of these preparations on ova of the same species and the results obtained are described first for *Arbacia*. The ova were exposed to the action of sperm filtrates and dialyzates in the proportion of two milliliters of fresh washed eggs to twenty-five milliliters of the test solution. Sea-water controls accompanied every experiment and samples of eggs from test solution and from sea-water were examined at intervals.

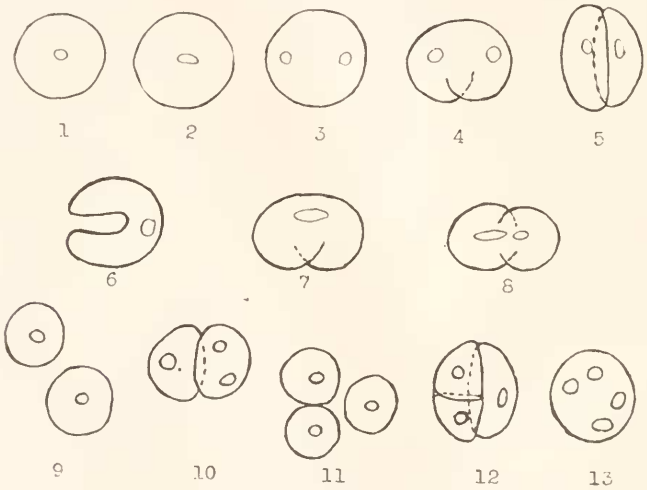
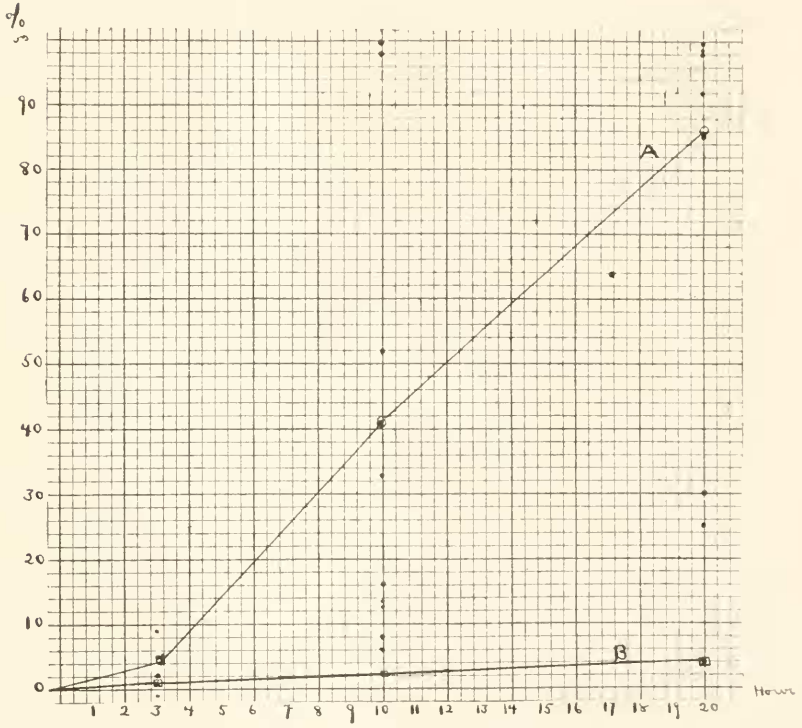


Fig. 2. Changes in shape and nuclear changes in ova exposed to sperm filtrates. 1–5 = changes in a single ovum within forty-five minutes; 6–13 = ova representing various changes produced by filtrates. Magnification × 350.

The first evidence of the influence of the filtrate or of the dialyzate on the ova is a change in the density of the cytoplasm at or near the center of the ovum, similar to that which precedes nuclear division in inseminated eggs. Indeed nuclear changes and nuclear division follow. Simultaneously a distortion of the egg occurs followed by its partial or complete cleavage into two or more parts. The cleavages are at times perfectly regular, at times unequal. Even the smallest cells are usually nucleated. Segmentation sometimes proceeds to the eight-celled stage, but in any case is followed, if the eggs remain in the filtrate, by a

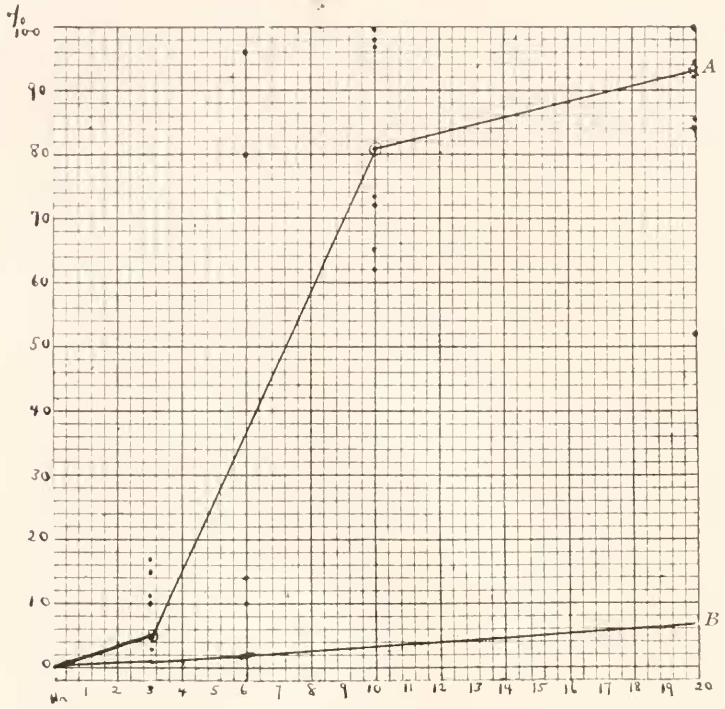
separation of the parts and their ultimate cytolysis. In a very few cases a slightly elevated membrane is visible. These results were reproduced with different lots of eggs and with different filtrates and dialyzates. In no case did these changes occur in sea-water controls or in sea-water filtered or dialyzed. The latter results indicate that no substance is given off by the filters or collodion sacs that can account for the effects of sperm filtrates and dialyzates.



GRAPH I. The percentage of ova divided and cytolyzed by sperm filtrates of the same species—*Arbacia punctulata*. *A* = the average of ten experiments with filtrates made from 2 per cent. suspensions. *B* = the average of ten sea-water controls. ● = percentages in individual experiments with sperm filtrates.

Confirmation of these results was obtained in a series of forty-two experiments with filtrates and twenty-four experiments with dialyzates. Within twenty-four hours the majority of ova in the test solutions had undergone decided changes, while those in

controls remained normal in appearance. This is well illustrated in the percentages indicated in graphs I., II., and III.[3]



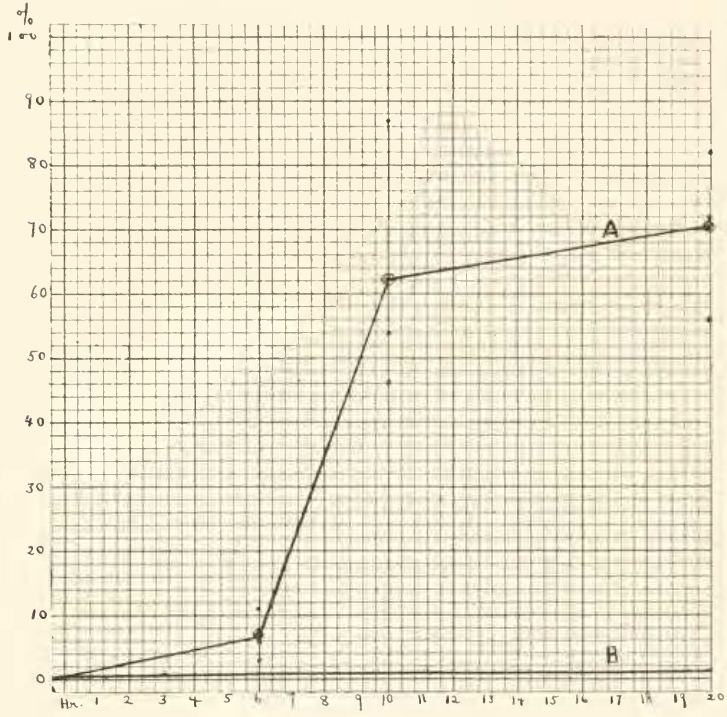
GRAPH II. The percentage of ova divided and cytolyzed by sperm filtrates of the same species—*Arbacia*. *A* = the average of ten experiments with filtrates made from 5 per cent. suspensions. *B* = the average of ten sea-water controls. ● = percentages in filtrates in individual experiments.

It is evident that some ova, probably because of a slight difference in physiological condition, are more rapidly affected by the filtrates and dialyzates than others, thus indicating a varying degree of susceptibility of the eggs to the action of the preparations.

In view of the failure of other investigators of this problem it is important to emphasize the fact that I obtained consistent results. At the same season of the year the eggs of different

[3] The graphs indicate the number divided and cytolyzed rather than simply the ones in a state of division. Since the ova do not divide simultaneously, some have divided and cytolyzed at a time when others are undergoing nuclear changes or are dividing.

females are about equally susceptible, and filtrates or dialyzates prepared in the same manner are equally effective on ova of the same individual, as indicated in graphs IV. and V.



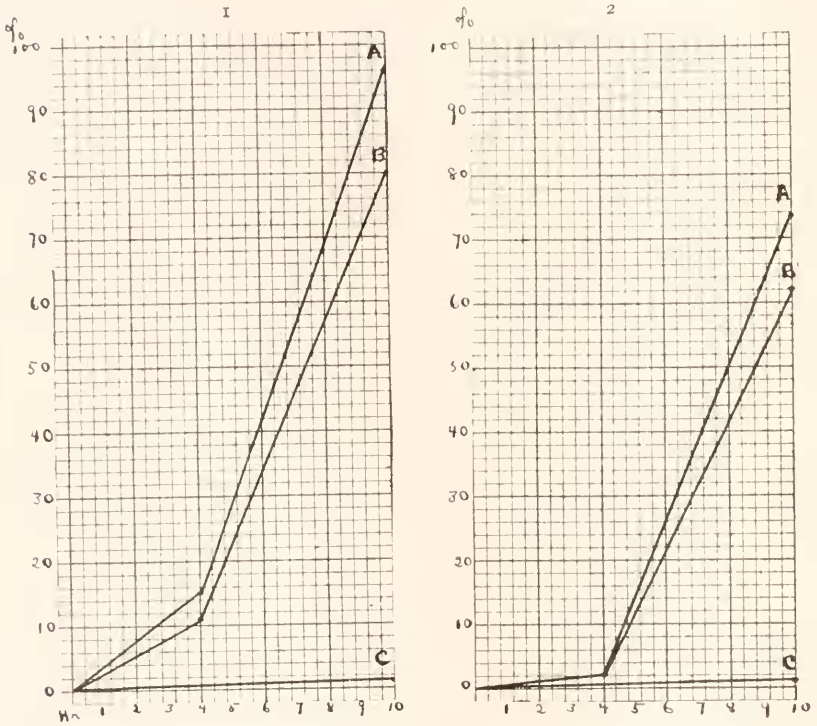
GRAPH III. The percentage of ova divided and cytolyzed in sperm dialyzates of the same species—*Arbacia punctulata*. *A* = the average of three experiments with dialyzates from 50 per cent. suspensions. Duration of dialysis = ten hours. *B* = the average of three experiments with sea-water dialyzed for ten hours against sea-water. ● = percentage in dialyzates in individual experiments.

It is apparent that, if the eggs are in the same physiological condition and are exposed at the same temperature to filtrates of equal strength, one may expect equal amounts of segmentation and cytolysis in approximately equal lengths of time.

As might be expected a definite correlation exists between the strength of the test solutions and the percentage of eggs affected in a given time. This is well illustrated in graphs VI., VII., VIII., and IX.

The definite correlation which exists between the duration of

exposure and the percentage of eggs affected suggested experiments in which the eggs are removed from the filtrate before the latter have had opportunity to produce any visible changes in the eggs. If now there are invisible effects and if these are orderly and significant, they should become noticeable after the

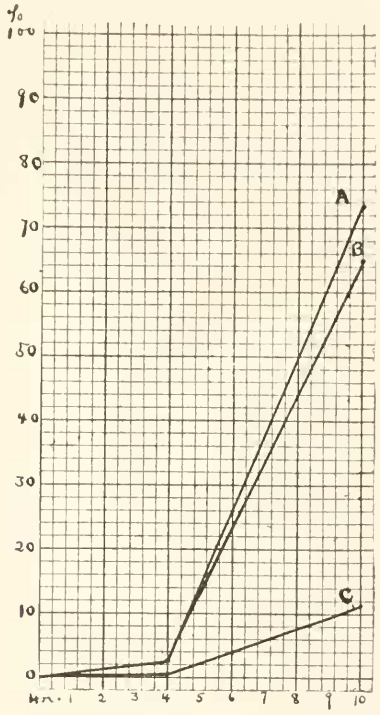


GRAPH IV. Percentage of division and cytolysis of ova produced by sperm filtrates on two sets of eggs—*Arbacia punctulata*. 1 = Filtrate 6/30/21 from a 5 per cent. suspension. 2 = Filtrate 7/2/21 from a 5 per cent. suspension. *A* = one set of eggs. *B* = second set of eggs. *C* = control eggs in S.W.

eggs are transferred to sea-water. Cases in point are illustrated in graphs X., XI., and XII., and indicate clearly that the gross visible effects, distinct only after several hours, are preceded by important changes which may develop in some of the ova within a very few minutes. These changes may, after transfer of the ova to sea-water, lead to progressive changes in the ova.

The Effect of Filtrates Prepared from Heated Sperm.—The statement was made by Winkler (1900) and by Morse (1912)

that when sperm suspensions were heated to 50°–60° centigrade the solutions failed to affect eggs. I therefore made filtrates from suspensions of *Arbacia* sperm heated to 42°–50° centigrade to compare their action with those of filtrates from unheated



GRAPH V. Percentage of ova divided and cytolyzed by two similar filtrates of sperm of the same species—*Arbacia*. *A* = Sperm filtrate 6/30/21 from a 5 per cent. suspension. *B* = Sperm filtrate 7/2/21 from a 5 per cent. suspension. *C* = Sea-water control.

sperm suspensions. The spermatozoa lose their iso-agglutinable and their fertilizing properties at a temperature of 38°–40° centigrade and tend to adhere to one another. One heated suspension was first passed through filter paper and then through a Mandler filter. As a control a part of the same suspension, unheated, was similarly treated. A comparison of the effect of the two filtrates is given in the following table.

TABLE II.

A COMPARISON OF THE ACTION OF FILTRATES PREPARED FROM HEATED AND FROM UNHEATED SPERM SUSPENSIONS OF *Arbacia* FIRST PASSED THROUGH WHATMAN FILTER PAPER.

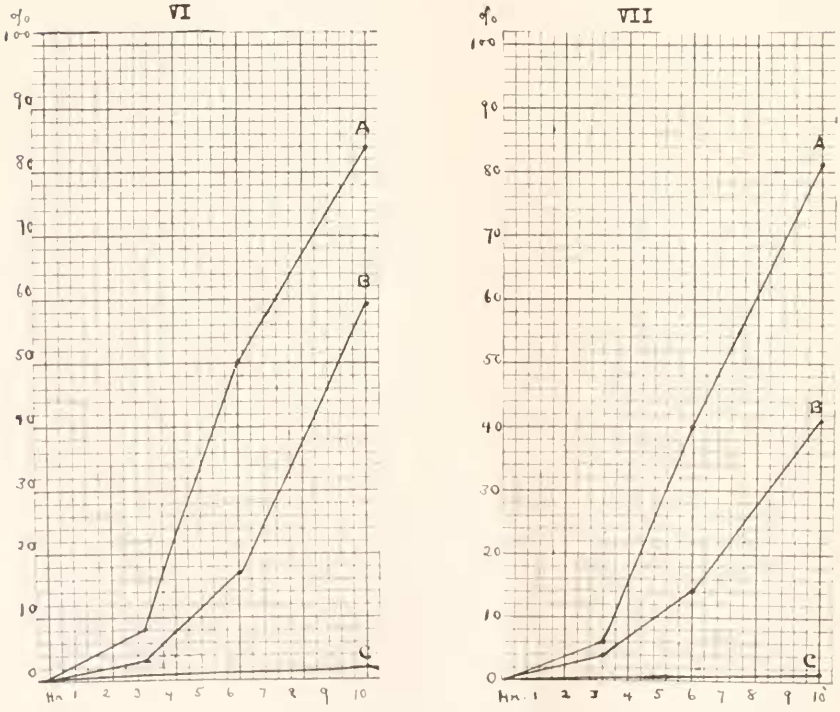
Filtrate from	Per Cent. of Ova Affected.				Per Cent. of Ova Affected.			
	4.5 Hrs.				7.5 Hrs.			
	Nor.	Abn.	Div.	Cyt.	Nor.	Abn.	Div.	Cyt.
I. Fresh Sperm	74	24.5	0.5	1	1	0	0	99
II. Heated Sperm	99	0	0	1	99	0	0	1

Nor.—normal undivided ova.

Abn.—undivided ova, abnormal in shape and in the appearance of the cytoplasm and nucleus.

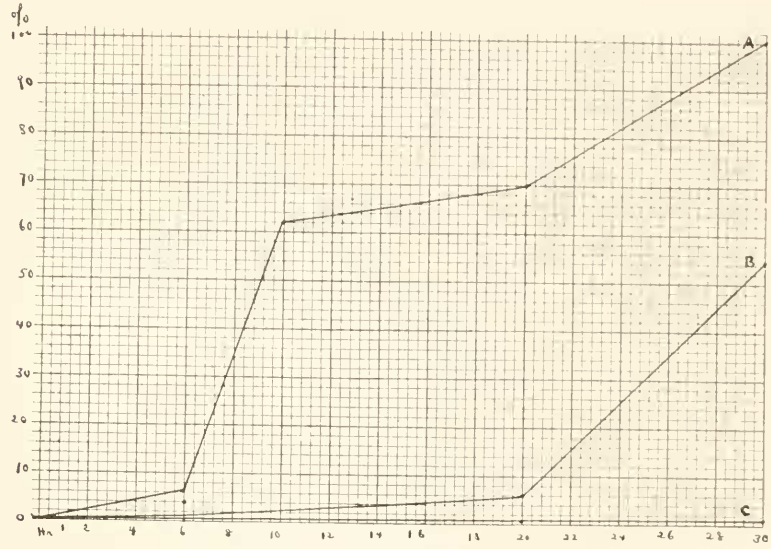
Div.—divided.

Cyt.—cytolyzed.



GRAPHS VI. AND VII. The relation of the strength of the filtrate to the percentage of ova divided and cytolyzed—*Arbacia*. VI. Average of four experiments. VII. Average of twelve experiments. *A* = sperm filtrate from a 5 per cent. suspension. *B* = sperm filtrate from a 2 per cent. suspension. *C* = sea-water control.

Practically no heated sperm reach the surface of the filter mantle as they do not pass through the Whatman filter paper. This might appear to indicate that any active substance is adherent to the heated coagulated sperm and not readily given off into sea-water.



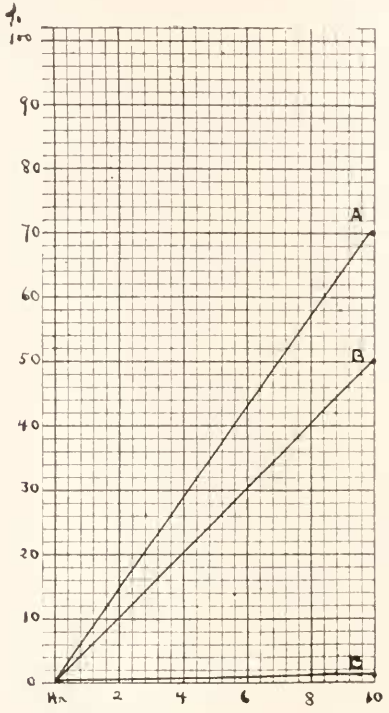
GRAPH VIII. Relation of the strength of the dialyzate to the percentage of ova divided and cytolyzed—*Arbacia punctulata*. *A* = average of three experiments with dialyzates from 50 per cent. sperm suspensions. *B* = average of three experiments with dialyzates from 25 per cent. sperm suspensions. *C* = average of six experiments with sea-water controls. Duration of dialysis = ten hours.

Another filtrate was made by filtering a heated sperm suspension directly through a Mandler filter. As a control part of the same suspension, unheated, was passed through a second filter. The effects of these two preparations are compared in Table III.

The filtrates of the heated sperm suspension have as great an effect on unfertilized ova of the same species as filtrates of fresh suspensions if the suspensions are filtered directly.

The Effect of Boiled Filtrates of Arbacia Sperm.—If the substance which so affects the eggs is an enzyme, heated filtrates may be ineffective. A filtrate prepared from a five per cent. sperm suspension was heated to boiling and then cooled rapidly.

An equal volume of sea-water was treated in the same manner and used as a control. Unheated filtrate and unheated sea-water served as additional controls. The results are given in Table IV.



GRAPH IX. Correlation between the duration of dialysis and the percentage of ova divided and cytolyzed—*Arbacia*. *A* = dialyzate from a 50 per cent. sperm suspension. Duration of dialysis = twenty hours. *B* = dialyzate from a 50 per cent. sperm suspension. Duration of dialysis = ten hours. *C* = sea-water dialyzed for twenty hours against sea-water.

Heating the filtrate as above indicated does not greatly affect its power to produce segmentation and cytolysis of ova of the same species. It should be emphasized that the hydrogen-ion concentration and the osmotic pressure of these solutions are like those of the sea-water controls.

It is thus evident that filtrates and dialyzates of spermatozoa of *Arbacia* contain some substance, not destroyed by heat, which produces profound changes in ova of the same species.

TABLE III.

A COMPARISON OF THE ACTION OF FILTRATES PREPARED FROM HEATED AND FROM UNHEATED SPERM SUSPENSIONS OF *Arbacia* FILTERED DIRECTLY THROUGH MANDLER FILTERS.

Exp.	Filtrate from	Per Cent. of Ova Affected. 2 Hrs.			Per Cent. of Ova Affected. 20 Hrs.		
		Nor.	Abn.	Cyt.	Nor.	Abn.	Cyt.
167	I. Fresh Sperm	98	0	2	0	0	100
	II. Heated Sperm	100	0	0	0	0	100
170	I. Fresh Sperm	62	0	38	0	0	100
	II. Heated Sperm	53	0	47	0	0	100

Nor.—normal undivided ova.

Abn.—undivided ova, abnormal in shape and in the appearance of cytoplasm and nucleus.

Cyt.—cytolyzed. (Partial or complete division precedes cytolysis.)

TABLE IV.

A COMPARISON OF THE ACTION OF HEATED AND UNHEATED SPERM FILTRATES ON OVA OF THE SAME SPECIES—*Arbacia punctulata*.

Time = 14 Hrs.	Per Cent. of Ova Affected. Exp. *A*—III.			Per Cent. of Ova Affected. Exp. *A*—IV.		
Solution.	Nor.	Div.	Cyt.	Nor.	Div.	Cyt.
Sea-water	98	0	2	99	0	1
Heated Sea-water	98	0	2	99	0	1
Sperm Filtrate	62	3	35	74	3	23
Heated Filtrate	84	0	16	81	2	17

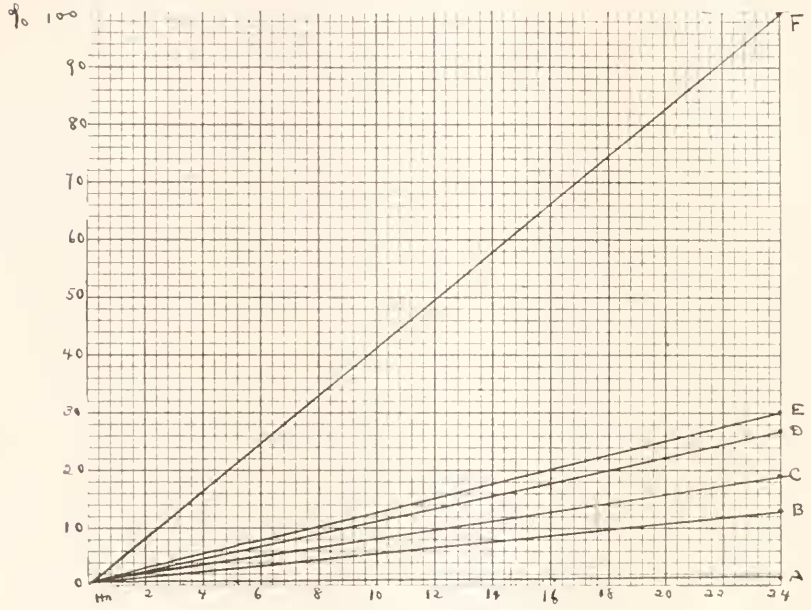
Nor.—undivided and normal.

Div.—divided.

Cyt.—cytolyzed.

b. Strongylocentrotus purpuratus and Strongylocentrotus franciscanus.—The same methods employed with two other species of sea-urchins—*Strongylocentrotus purpuratus* and *Strongylocentrotus*

franciscanus—at Pacific Grove, California, from January to June 1921, yielded similar results. Longer exposures were required than with ova of *Arbacia*. The ova of *S. franciscanus* were the more susceptible to the action of the sperm filtrates, but unfortunately ripe ova of this species were rare during these months



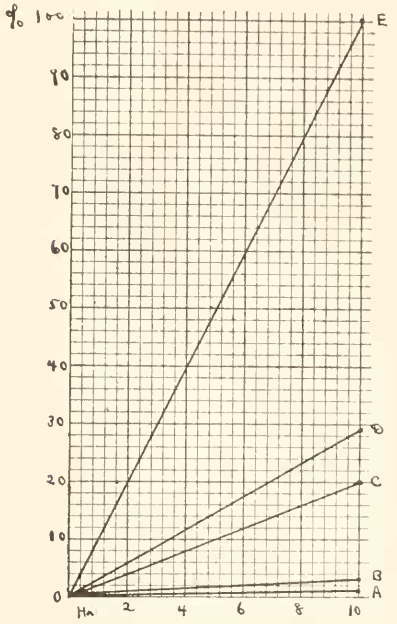
GRAPH X. The effect of limited exposure of ova to sperm filtrates—*Arbacia*. Experiment 156—Observation at the end of twenty-four hours. The percentage of ova divided and cytolyzed is indicated. *A* = sea-water. *B* = exposure to 5 per cent.* filtrate for five minutes. *C* = exposure to 5 per cent. filtrate for ten minutes. *D* = exposure to 5 per cent. filtrate for thirty minutes. *E* = exposure to 5 per cent. filtrate for sixty minutes. *F* = exposure to 5 per cent. filtrate for twenty-four hours.

of 1921 because of an extremely cold season. It is well known that the ova of *S. purpuratus* are resistant to parthenogenetic agents, Loeb (1916, pp. 99–103). The hope that the conditions of low temperature (12°–15° C.) and high hydrogen-ion concentration (p_H 7.6–7.8) prevailing at Pacific Grove might favor normal segmentation and normal development of ova treated with sperm filtrates was not realized. Yet, as will be demonstrated in later experiments, a brief exposure to such filtrates

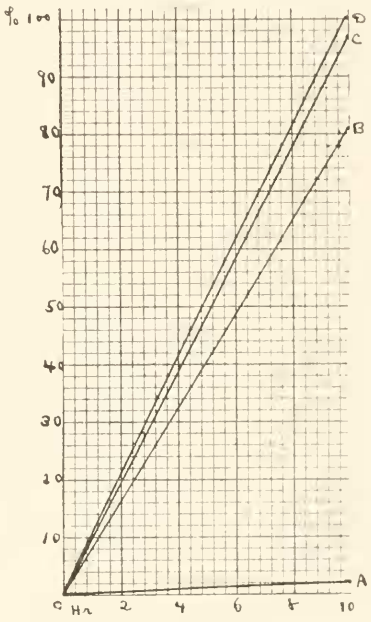
* 5 per cent. filtrate = a filtrate from a 5 per cent. suspension.

produced decided changes in ova of both species of *Strongylocentrotus*.

c. Nereis limbata.—Sperm filtrates of *Nereis limbata* produce effects on ova of this species comparable to those produced by foreign sperm filtrates. An exposure of *Nereis* eggs to filtrates



GRAPH XI.



GRAPH XII.

The effect on ova of *Arbacia* of limited exposure to filtrates.

Filtrate of a 2 Per Cent. Suspension.	Filtrate of a 2 Per Cent. Suspension.
A = sea-water 10 hrs.	*A* = sea-water 10 hrs.
= filtrate 15″–45″	= filtrate 15″–45″
B = " 75″	*B* = " 210″
C = " 135″	*C* = " 4.5 hrs.
D = " 195″	*D* = " 9 hrs.
E = " 9 hrs.	

The percentages of ova divided and cytolyzed are indicated.

made from one, two or ten per cent. *Nereis* sperm suspensions results in the formation of jelly and fertilization membranes and the complete maturation of a certain percentage of eggs. The most satisfactory results were obtained with eggs transferred directly to a two per cent. filtrate, and within two hours after

the *Nereis* were collected. In this experiment the majority of the eggs matured. The maturation was almost as rapid as in fertilized eggs but the subsequent segmentation and development was slow and abnormal. Segmentation into two, four, and eight cells was observed and verified for me by Dr. Alvalyn Woodward. Free swimming trochophores developed which were abnormal in shape, internal structure, and in the distribution of cilia. In no case did such changes occur in the sea-water controls. Similar results were obtained with four sperm filtrates of *Nereis limbata*.

Summary of the Effects of Sperm Filtrates and Dialyzates on Ova of the Same Species.—Sperm filtrates and dialyzates of *Arbacia punctulata*, and sperm filtrates of *Strongylocentrotus purpuratus*, of *Strongylocentrotus franciscanus*, and of *Nereis limbata* produce marked changes in ova of the same species. These are characterized by changes in form; nuclear and cell division; and, with the exception of *Nereis limbata*, by subsequent fragmentation and cytolysis. "Fragmentation" is a separation of the segments due possibly to a lack of a fertilization membrane. In *Nereis limbata* the sperm filtrates cause maturation; formation of fertilization membranes; segmentation; and, in a low percentage of cases, the development of abnormal larvæ.

V. Filtrates as Factors in Parthenogenesis.

It seemed possible in view of the results obtained by Loeb (1905) and others that the ova of sea-urchins, treated with filtrates of sperm, might develop perfectly if they were subsequently exposed for a brief period to Loeb's "hypertonic sea-water". In Loeb's experiments, exposure of unfertilized ova of sea-urchins to hypertonic sea-water, at the temperature prevailing during the breeding season, produced development in a very small percentage of the ova. Fertilization membranes did not form usually and the developing plutei did not swim at the surface of the water as did normal plutei. A brief preliminary treatment of the ova with a very dilute solution of butyric acid increased the percentage of ova which developed, and the latter were normal in macroscopic structure and in their reactions. Recently Just (1922), employing a greater concentration than

that used by Loeb, has succeeded in producing healthy viable plutei of *Arbacia punctulata* by exposing ova of this species to hypertonic sea-water alone. In view of these results it may be that the butyric acid sensitizes the eggs to the action of the hypertonic sea-water. Experiments were undertaken to determine whether or not sperm filtrates may also act as sensitizers.

The procedure employed in this phase of the investigation was as follows: unfertilized eggs of *Arbacia* were exposed for periods of one minute to two hours to a sperm filtrate and subsequently for twenty to thirty-five minutes to Loeb's hypertonic sea-water. A control series consisted of ova exposed first to sea-water and then to Loeb's hypertonic sea-water. One set of unfertilized and one set of fertilized eggs served as controls for each experiment.

In the majority of experiments no development took place in the ova exposed to hypertonic sea-water only. When such an exception occurred, the percentage of ova which developed was small and the plutei did not swim at the surface of the water. In every experiment many of the ova subjected to the double treatment, *i.e.*, to the filtrate followed by the hypertonic sea-water, developed as far as the blastula and gastrula stages. A small percentage developed into perfect plutei which swam at the surface. The majority cytolyzed. Because of the high percentage which cytolyze at the time when the blastulæ and gastrulæ form, it is impossible to determine the exact percentage which develop to these stages. The sea-water containing them was centrifuged and the residue examined. An effort was made to ensure the transfer of equal quantities of eggs and of sea-water in each experiment, and equal periods of centrifuging were employed to ensure equal possibilities of precipitation of ova or of developing individuals. The results of these experiments are given in Table V.

As already indicated the sperm filtrates rarely cause membrane formation. In the few instances where these form, they are thin and but slightly elevated. In one filtrate such membranes developed on ova after an exposure to the filtrate for thirty to thirty-five minutes. Of the ova remaining in the filtrate for one to fifteen minutes and subsequently transferred to hypertonic

sea-water for twenty to thirty-five minutes, a larger percentage developed than in other experiments. In this series no ova developed after exposure to hypertonic sea-water alone. In Table VI. the results of these experiments are summarized. The figures indicate approximate percentages.

Table V.

A Comparison of the Number of Ova of *Arbacia* which Develop after Treatment with Filtrate Followed by Exposure to Hypertonic Sea-water.

Time of observation and total count = 70 hrs.

Transfer from	Sea-water.			Hypertonic S.W. 30″			Hypertonic S.W. 35″		
	B.	G.	P.	B.	G.	P.	B.	G.	P.
Sea-water 15″	0	0	0	1	0	1	2	0	0
Filtrate									
2″	0	0	0	13	0	5	56	0	28
10″	0	0	0	0	0	2	41	0	14
15″	0	0	0	74	0	0	36	0	3

The letters B, G, and P indicate blastulæ, gastrulæ, and plutei respectively. The figures given here indicate the number, not the percentage, of developing ova.

A comparison of the effects of the preliminary treatment with sea-water and with sperm filtrates reveals, in the latter experiments, a slight increase in the percentage of ova which develop into plutei perfect in macroscopic appearance and in reactions; and a decided increase in the percentage which cytolyze after exposure to hypertonic sea-water. The preliminary treatment with the sperm filtrate apparently sensitizes the ova to the action of the hypertonic sea-water.

Tests were also made to determine whether the sperm filtrates increase the susceptibility of the ova of *Strongylocentrotus purpuratus* to the action of hypertonic sea-water. Certain preliminary experiments were performed to familiarize the writer with the effect of hypertonic sea-water alone on the ova of this species. Ova were exposed to it for periods of thirty minutes to four hours. The effect was tested by transferring them to sea-

Table VI.

The Effect Produced on Ova of *Arbacia punctulata* by Sperm Filtrates of the Same Species Followed by Hypertonic Sea-water.

Transfer from	To Hypertonic S.W. 20″.				Hypertonic S.W. 25″.				Hypertonic S.W. 30″.				Hypertonic S.W. 35″.			
	Nor.	Div.	Dev.	Cyt.	Nor.	Div.	Dev.	Cyt.	Nor.	Div.	Dev.	Cyt.	Nor.	Div.	Dev.	Cyt.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Sea-water 15″	95.8	0	0	4.2	91	1	0	8	90	0	0	10	90	0	0.5	9.5
Filtrate 1″	75.5	0	0	24.5	53.6	0	5.7	41.7	75	0	4.5	20.5	27.5	0	1.5	71
5″	0	0	0	100	75	0	3.8	21.2	62	0	3	35	28.3	0	2	69.7
10″	66	0	3.4	30.6	8.8	0	0	91.2	21.8	0	6.1	72.1	23.6	0	0.4	76
15″	48	0	2.7	49.3	14	0	1	85.0	3	0	0	97	1	0	0	99

water and to inseminated sea-water. Ova treated for thirty minutes to two hours were uninjured. Longer exposures caused cytolysis of ova transferred to sea-water and prevented normal fertilization and development of ova subsequently inseminated in sea-water. The percentage of the ova affected bore a direct relation to the duration of the exposure to the hypertonic sea-water.

A repetition of Loeb's method of producing parthenogenesis in this species demonstrated that treatment with 1.5 per cent. butyric acid for one minute followed by hypertonic sea-water for one hour led to development of the majority of the ova. Other experiments indicated that a slight decrease in hydrogen-ion concentration (*e.g.*, p_H 8.3–8.7) facilitated the process. In place of the butyric acid ova were given a preliminary treatment with sperm filtrates in the hope of obtaining development, or of intensifying the action of the hypertonic sea-water. The results at the end of eighteen hours are indicated in Table VII.

Table VII.

Effect of Exposure to Filtrate Followed by Hypertonic Sea-water on Ova of *Strongylocentrotus purpuratus*.

Exp.	Prep.	% Filt.	Duration of Exposure.		Effects on the Ova.			
			To		Percentage.			
			Filtrate	Hypertonic S.W.	Nor.	Abn.	Div.	Cyt.
83	1/26	10	0″	70″	99	0	0	1
			30″	70″	98	0	0	2
			60″	70″	98	0	0	2
			120″	70″	66	0	1	33
			30″	120″	99	0	0	1
			60″	120″	0	97.5	0	2.5
			120″	120″	0	0	0	100

It has already been mentioned that ova of *Strongylocentrotus purpuratus* are more resistant to parthenogenetic agents than are those of *Arbacia punctulata*. In these experiments ova of the former species, exposed either to hypertonic sea-water alone

or to filtrate and hypertonic sea-water, failed to develop. Occasionally atypical membranes formed after the double treatment and irregular division ensued. It is of interest that ova which had been exposed to the filtrate for two hours did not shrink when transferred to hypertonic sea-water, as did those transferred directly from sea-water to the hypertonic sea-water. This would seem to indicate that the permeability of the ova exposed to the filtrate had increased. Another indication of the effect of the filtrate in these experiments follows: Ova given the double treatment of filtrate followed by hypertonic sea-water were less capable of normal fertilization and development than those exposed to hypertonic sea-water alone.

Summary of the Function of Filtrates as Factors in Parthenogenesis.—Exposure of ova of *Arbacia punctulata* or of *Strongylocentrotus purpuratus* to sperm filtrates of the same species produce changes which render them more susceptible to the action of hypertonic sea-water. This is indicated in *Arbacia* by a larger percentage of development and of cytolysis; and in *S. purpuratus* by a greater tendency of ova to cytolyze or to lose their power of normal fertilization and development if exposed to both filtrate and hypertonic sea-water.

VI. Filtrates as Factors in Fertilization.

The increase produced by sperm filtrates in the susceptibility of *Arbacia* ova to parthenogenetic agents, and the apparent increase in permeability of ova of *S. purpuratus* suggest that such preparations may also facilitate fertilization.

An attempt was made to obtain "resistant" eggs early in the breeding season in order to try the effect of fertilizing such ova in sperm filtrates. During two seasons such "resistant" eggs were not found. The ova obtained were either immature, as indicated by their failure to develop if fertilized with a small or large amount of sperm suspension, or else ripe. In the latter case no increase over the usual percentage of development could be expected.

Conditions leading to increase in permeability of mature ova tend to allow the entrance of more than a single spermatozoön when such eggs are inseminated. This polyspermy usually

results in abnormal development. It may occur if mature ova are inseminated in sperm filtrates. To determine this, two milliliters of washed *Arbacia* eggs were placed in twenty-five milliliters of sperm filtrate and, as a control, a similar quantity in twenty-five milliliters of sea-water. These were inseminated immediately, or after exposure to the filtrate or sea-water for from one minute to four hours. Frequent observations were made to determine whether the filtrates affect either fertilization or development. The effects observed are as follows in the ova fertilized in the filtrate and allowed to develop in it: lack of a normal fertilization membrane; abnormal changes in shape; a low percentage of dividing ova; abnormal division; delayed and abnormal development; and cytolysis. In the controls normal fertilization and development occurred. Similar results were obtained in thirteen series of experiments in which several filtrates were used. Gemmil (1900) describes similar conditions resulting from heavy insemination and finds them accompanying polyspermy as demonstrated by histological examination.

The abnormal development which occurs when ova are inseminated in the sperm filtrates may be a result of an abnormal fertilization reaction. If this is due to changes in the ova in the filtrate, it may be that such changes are reversible if the exposure to the filtrate is of short duration. Ova of *Arbacia punctulata* were exposed to filtrates made from two per cent. suspensions for from one minute to two and a half hours, and to filtrates from five per cent. suspensions for one to thirty minutes. Some were inseminated in the filtrates and others after transfer to sea-water. Few of those inseminated in the filtrate developed normally. The majority of those inseminated after transfer to sea-water formed fertilization membranes and developed normally. The changes produced in the ova by the filtrates, which tend to prevent normal reactions between ova and sperm, are reversible if the period of exposure is brief. Prolonged exposure to filtrates, however, wrought such changes in the majority of ova that they lost their capacity for fertilization and development completely.

Ova of *Strongylocentrotus purpuratus* exposed for short periods to sperm filtrates may not exhibit any change in optical appear-

ance or in shape yet may be so influenced that after transfer to sea-water they fail to develop normally if inseminated with fresh sperm. Such was found to be the case in eighteen experiments in which ova were exposed to the action of a number of filtrates and subsequently inseminated in sea-water. Although the sperm were active in the filtrates, few membranes formed and these were abnormal in that they were irregular and but slightly raised from the surface of the egg. Subsequent divisions were irregular; development was slow and abnormal; and the majority of ova cytolyzed later. Gastrulæ formed in a few, but these lacked an enteron, and plutei were irregular in shape with thickened areas not normally present. These resembled plutei obtained when ova are inseminated with a large excess of sperm when polyspermy is known to occur.

A correlation between the reversibility of changes in ova produced by abnormal constituents in sea-water and the duration of their exposure to these substances has been recorded by Loeb (1915) for butyric acid and hypertonic sea-water; by Lillie (1921) for copper; and by Clowes and Smith (1923 and 1924) for hydrogen ions. In some instances the change is of such a nature that it acts as a block to the entrance of sperm; in others it permits polyspermy. Just (1923) found that eggs of *Echinarachnius* fertilized in blood, though they fail to develop, nevertheless take in sperm.

As stated by Clowes (1924) "it is difficult to distinguish polyspermic from abnormally dividing eggs without cytological examination." Such a study of ova of *Arbacia* inseminated in the sperm filtrates reveals that polyspermy occurred in many of the ova.

The interference with the fertilization process may be due in part to injury to the sperm caused by the sperm filtrate. However, examinations of suspensions of sperm in filtrates reveal that the sperm remain active in such suspensions for hours. Furthermore if such sperm are used for insemination of ova in sea-water, they will effect normal fertilization and development. The results tabulated below indicate that the sperm are not injured by an exposure of one hour either to sea-water or to a sperm filtrate in the concentrations employed; but that ova

were affected by a similar duration of exposure to some of the same filtrate.

TABLE VIII.

A COMPARISON OF THE ACTION OF FILTRATES ON THE FERTILIZING CAPACITY OF SPERM AND OF OVA OF *Strongylocentrotus purpuratus*.

Exp.	Prep.	Filt.	Expos.	Ova from Sea-water.				Ova from Sperm Filtrate.			
73	12/28/11	10%		% Nor.	% Div.	% Abn. Div.	% Cyt.	% Nor.	% Div.	% Abn. Div.	% Cyt.
Sperm exposed to sperm filtrate..			0″	0	90	10	0	0	90	10	0
			1″	0	94	3	3	0	59	35	6
			5″	0	95	5	0	0	85	10	5
			10″	0	91	5	4	0	68	29	3
			20″	0	95	3	2	0	56	43	1
			30″	0	96	3	1	3	23	18	56
			60″	0	98	2	0	0	0	30	70
Sperm suspension in sea-water....			30″	0	98	1	1	0	0	33.4	66.6
			60″	0	96	0	4	0	0	12	88

It is also possible that filtrates may interfere with the development of fertilized ova. This proved to be true. The ova of *Arbacia punctulata* and of *Strongylocentrotus purpuratus* transferred ten minutes after insemination in sea-water to sperm filtrates failed to develop normally, and within eighteen hours the majority had cytolyzed.

Summary of the Action of Filtrates as Factors in Fertilization.— Sperm filtrates produce changes in the eggs of the same species which interfere with a normal fertilization reaction if the eggs are inseminated in the filtrate. The changes produced by a brief exposure are reversible; by a longer exposure irreversible. Such filtrates prevent normal development of eggs previously inseminated in sea-water. The changes are of such a nature that the entrance of sperm is facilitated and polyspermy results.

VII. TEST OF FILTRATES FOR A "SPERM-FERTILIZING AND AGGLUTINABLE SUBSTANCE."

Lillie (1919) has suggested that the spermatozoa bear a fertilizing substance identical with the agglutinable substance

which is apparently lost by spermatozoa in staling in sea-water. If this is present in sea-water filtrates and dialyzates, it should combine with the "agglutinin" of "egg-water." This was tested as follows. The agglutinating unit strength of *Arbacia* egg-water was first determined, Lillie (1914). Dilutions of such egg-water with sperm filtrates and with sea-water were compared as to their power to agglutinate fresh sperm suspensions and no difference could be detected in the capacity of the two sets of dilutions to agglutinate sperm. Either an insufficient amount of the combining substance is present in the sperm filtrates or the substance or substances present do not have the power to combine with the agglutinin of the egg-water.[4]

The agglutinin does not pass through collodion sacs. If the substance in sperm dialyzates is the substance postulated by Lillie, egg-water dialyzed against a sperm suspension should lose its agglutinating power more rapidly than a similar egg-water dialyzed against sea-water. In experiments devised to test this theory no difference in the rate of loss of agglutinin could be detected.

The "fertilizin" of Lillie in *Arbacia* egg-water may, according to Woodward (1918), consist of two parts: an agglutinating and an activating substance. The latter only passes through a Mandler filter. This activates sperm of the same species, and has the power of causing parthenogenetic development of ova of *Nereis limbata*, Sampson (unpublished, quoted by Woodward) and Woodward (1921). If its action is intensified by a fertilizing substance given off by sperm, a combination of filtrates of sperm and of egg-water should be more effective than either alone. No such intensifying effect could be demonstrated in any of the experiments devised to test this possibility.

VIII. Discussion.

In *Nereis limbata* and in sea-urchins the fertilizable period of the gametes is short. Causes for the brevity of this period have been discovered for ova but not for spermatozoa, as indicated by Lillie and Just (1924) in their recent survey of the subject.

[4] It should be recalled that some of the sperm remaining on the surface of the filter mantles and in the dialyzing sacs are agglutinable and retain their fertilizing power at the end of the periods of filtration and of dialysis.

The fact that loss of fertilizing power of the sperm occurs rapidly and precedes loss of motility has suggested the idea that the spermatozoön carries a fertilizing substance which may be lost in sea-water; and this has led to various attempts, of which this is the most recent, to isolate such a substance and to produce development of ova of the same species with it. In this investigation filtrates and dialyzates of sperm suspensions in sea-water have been obtained which initiate development of specific ova, although the development is incomplete.

It is not surprising to find that concentrated sperm suspensions exposed to egg-secretions of a foreign species, that extracts of cells, egg-secretions, and blood cause cytolysis of alien ova, "since it is recognized that something present in mammalian blood serum cytolyzes cells of unrelated animals," Loeb (1913). Sperm killed by heat, extracts of cells, and blood serum have no activating effect on ova of the same species. Specific egg-secretion ("egg-water") has no effect on ova of *Nereis*, but according to Glaser (1915) and Woodward (1918) does produce incomplete activation of ova of *Asterias* and *Arbacia*. Prolonged exposure of ova is necessary, and Lillie and Just (1924) have suggested that there are extraneous parthenogenetic factors present in the egg-water employed.

Careful tests of all the sperm filtrates and dialyzates used in this investigation indicate that the preparations do not contain living sperm or fragments of them. The ova employed are not normally parthenogenetic. Controls give evidence that no substance derived from filters or dialyzers is accountable for the results obtained; and factors which might produce parthenogenesis: abnormal specific gravity, abnormal hydrogen ion concentration, and excess of carbon dioxide, are lacking. The preparations contain carbon but insufficient nitrogen to be detected even by microchemical methods. No lipolytic enzyme could be detected. The preparations are not colloidal; and all attempts to obtain precipitates from them by means of alcohol or the reagents used by Robertson (*loc. cit.*) and Woodward (*loc. cit.*) failed. Sperm filtrates and dialyzates activate ova rapidly and this property is not destroyed by boiling. The effect produced is evidently due to a special physiological activator derived from sperm of the same species.

Experiments to determine whether the substance or substances present in filtrates and dialyzates are "tissue specific," acting only on ova, have not been undertaken. It is true that they do not cytolyze species sperm, as the latter retain their fertilizing power after prolonged exposure to such preparations. The latter are not "species specific" since they readily activate ova of unrelated species. Tests have not been performed to determine whether the effect on species and on foreign ova is due to the same constituent of the preparations.

If the substance acts as a superficial cytolytic agent as suggested by Loeb (1916), it is to be expected that membrane elevation or swelling will occur in ova of the same species exposed to the sperm filtrates and dialyzates. Such occurred in *Nereis* only. However, such preparations cause partial activation and changes in the protoplasm of sea-urchin ova; and there is evidence that the properties of the egg surface are affected. Thus, after exposure to sperm filtrates, unfertilized ova of sea-urchins are more susceptible to the action of hypertonic sea-water; are in a condition which facilitates polyspermy; and their permeability is increased. Fertilized eggs, transferred to such preparations within ten minutes after insemination in sea-water, fail to develop normally. This may be due in part at least to an increase in the permeability of the egg surface. The sperm are uninjured by long exposure and the changes in the ova are reversible if the duration of exposure to such preparations is brief.

According to the "fertilizin" theory of Lillie (1914), substances ("receptors") given off by sperm activate "fertilizin" an essential constituent of the cortex of mature eggs. This in turn initiates the development of the egg. If "receptors" exist in active form and in sufficient quantity in these sperm filtrates and dialyzates, the latter should produce the following effects: initiation of development of mature ova of the same species; such activation of "fertilizin" in egg-water as to make the latter an efficient parthenogenetic agent; such combination with an agglutinating substance in egg-water as to destroy the power of the latter to agglutinate fresh sperm suspensions. The first of these results only has been obtained in this investigation. If

sperm receptors are released more readily in specific egg-water, filtrates of sperm suspensions in egg-water may evince greater activating power than a combination consisting of a sperm filtrate and filtered egg-water. Tests gave no indication of any difference between them. It is also possible, as suggested by Lillie (1915), that other substances are extracted from sperm which may tend to neutralize the activating substances released by them.

There is abundant evidence that the sperm in contact with mature ova, or secretions of ova, of the same species undergo changes which are essential for fertilization. Their metabolism is increased as indicated by their increased motility; their chemical composition is changed as indicated by decrease in refringibility, by swelling of the sperm head, by changes in viscosity, and by surface changes which permit agglutination to occur. Spermatozoa may enter unripe eggs which lack "fertilizin" or mature eggs from which it has been removed experimentally but the sperm are not changed and they do not activate the eggs.

It is significant that in order for fertilization to occur *these essential changes in the spermatozoön must be produced when the latter is in close proximity to an ovum.* Sperm which have received a long exposure to sea-water or a brief exposure to egg-water are active and may surround or even enter ova of the same species, yet fail to fertilize them. This loss of fertilizing capacity, the transitory nature of agglutination, and the inability to obtain a second agglutination reaction with sperm are indications of a loss of substances essential in fertilization. To effect perfect development *such substances must be concentrated at the surface of the sperm head at the instant when the latter comes into contact with an ovum.* Under such conditions they may initiate a chain of chemical reactions, starting in the cortex of the ovum and eventually involving all parts of the protoplasm of the egg, *i.e.*, they may activate "fertilizin." If released into sea-water or egg-water normally, or under the experimental conditions of filtration and dialysis, these substances may be unable to produce complete activation of ova of the same species, because of dilution, instability, or neutralization by other substances elimi-

nated or extracted from sperm. They may, however, produce changes in the surface of the ovum which facilitate its reaction with a spermatozoön. These changes may account, in part, for polyspermy when ova are inseminated with an excess of fresh sperm and for the effectiveness of stale sperm if concentrated suspensions are used. Such substances may account for the antagonistic effect produced by sperm of one species on those of another; and for the neutralizing effect of concentrated sperm suspensions on the action of blood serum.

IX. Summary.

1. Solutions obtained by filtration and dialysis of suspensions of living sperm in sea-water activate ova of the same species.

2. Tests indicate that the effect is produced by some substance derived from the sperm, and not by some extraneous parthenogenetic factor.

3. Ova of *Nereis* exposed to specific sperm filtrates form fertilization membranes, complete their maturation and some develop into abnormal trochophores. Ova of sea-urchins fail to form membranes but do undergo nuclear and cell division.

4. Ova exposed to filtrates and dialyzates are rendered more susceptible to the action of hypertonic sea-water, and to the entrance of sperm.

5. In normal fertilization sperm exposed to "fertilizin" undergo profound modification in chemical structure and organization; and unless such modification occurs the sperm fail to fertilize the egg, even though they may enter it. It is possible that substances localized in the surface of the sperm head activate the ovum. Such localization is transitory.

6. Such substances, when given off by sperm into sea-water or egg-water, either because of dilution, decomposition, or admixture with waste products given off by sperm, produce definite changes in such ova, but are unable to effect complete activation of ova of the same species.

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IS THE INSECT METAMORPHOSIS INFLUENCED BY THYROID FEEDING?

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(With 5 Tables.)

The investigations on the influence of the thyroid gland on the rate of metamorphosis in insects have not as yet yielded any decisive results. Northrop ('17) fed larvæ of *Drosophila* on thyroid and ascertained that this gland is not adequate as food for the examined animals. Kunkel ('18) who studied the influence of the thyroid on the development of the fly *Lucilia*, noticed an abbreviation of the pupal stage and a retardation of growth. Abderhalden ('19) irrigated *Euphorbia* plants with 1 per cent. thyroid extract and observed that the caterpillars of *Deilephila euphorbiæ* fed on these plants were "zum Teil auffallend klein." In the researches of Romeis and v. Dobkiewicz ('20) the larvæ of the flies *Calliphora vomitoria* fed on thyroid gland underwent pupation later than the control specimens, their weight, however, being not smaller. From his experiments Kahn ('21) draws the conclusion that in *Corethra plumicornis*, *Ecdyurus forcipula* and *Tenebrio molitor* neither the human thyroid nor the preparation "Jodalbacid" have any influence on the metamorphosis of their larvæ.

The discrepancy of the above results may be explained not only by the heterogeneity of the material of animals and of the substances used, but, at least to the same degree, by the probably too scanty material in separate experiments as well as by the insufficient method of the elaboration of the data recorded. *E.g.*, Abderhalden's opinion mentioned above is not supported by any numerical data and can consequently not be considered as decisive from the biometrical standpoint, all the more as we do not know the pedigrees of the animals under examination.

[1] Paper from the Laboratory of Experimental Morphology., cf. *Mém. de l'Institut National Polonais d'Économie Rurale à Pulawy*, Vol. 5, 1924, presented at print September 22, 1924.

Similarly, we cannot, according to my view, consider as decisive the results obtained by Kahn who relies on 16 *Tenebrio* larvæ of which 6 were kept without any food, and every two specimens from the remaining 10 were fed differently (fresh meat, fresh thyroid, dried thyroid, meal and "Jodalbacid"). The experiments of the same author on *Corethra* and *Ecdyurus* were made on specimens collected in natural waters and therefore on genetically totally unknown materials. Similar relations are often met with also in other problems referring to the glands of internal secretion. In these cases genetically homogeneous animals used for the experiments and statistical elaboration of adequate data, would, according to my opinion, contribute to set aside numerous discrepancies.

My experiments have been made on caterpillars from 4 lots of eggs of *Lymantria dispar* L. deriving from 4 brother-sister matings of moths belonging to the same pedigree and reared by the author for several years. In order to deal with materials homogeneous also in respect to age, I selected exclusively caterpillars which hatched during the night between April 27th and 28th. On June 5th the thoroughly mixed caterpillars of each lot were divided at random in two parts, one of which served as control, the other being fed on leaves with thyroid. Fresh willow twigs were accurately sprinkled with water solution of the "Tablettæ thyreoideæ" from the factory of "Gedeon Richter" in Budapest. Ca. 450 experimental caterpillars received daily: in the beginning 2, from June 12th 3, from June 18th 4 tabloids, equal to 1.0, 1.5 and 2.0 grams respectively of fresh gland. (The efficacy of the preparation had been previously tested on tadpoles of *Rana temporaria*.) The control animals were fed on willow twigs of the same species, sprinkled with pure water. In both cases the leaves were administered daily (after the superfluous water had been dried) in slight excess of requirements, so that the whole food was almost totally consumed by the next day. The thyroid-fed caterpillars took food as readily as those of the control.

All animals were bred in pasteboard boxes of identical size at a temperature of from 14° to 19° C. Each chrysalid being kept separately, the duration of the pupal period could be established

for each specimen. Pupation as well as emergence of moths was checked every day between 6 and 6:15 o'clock in the evening. The chrysalids were weighed immediately afterwards, so that the age of the weighed pupæ ranged from 0 to 24 hours. The data obtained were biometrically studied according to the commonly used formulæ. On account of the remarkable sexual dimorphism the data were collected for either sex separately. The total number of pupæ was 818, of which 234 male and 188 female were thyroid fed specimens and 171 male and 225 female control animals. Emergence occurred from 782 chrysalids, viz. 218, 182, 161 and 221 specimens respectively (cf. the number of animals in separate experiments in Tables I. and II).

The first chrysalis of the whole material appeared on June 24th. The average terms of pupation of the thyroid-fed and of the control caterpillars calculated in relation to the above term of the "first chrysalis" are recorded in Table I. We notice that the observable differences of the terms of control and experimental material were positive only in 3 cases, in the remaining 5 being on the contrary negative. The difference was here biometrically significant exclusively in the males of the second experiment, the ratio of the difference to its probable error in this case only approximating the required number 4. Moreover, on surveying the data referring to the duration of the pupal period (Table II.) we observe that here too the difference between the control and the thyroid-fed animals is not always positive, the ratio of the difference to their probable errors oscillating only between 0.05 and 2.5 and therefore being in no case significant. This indicates that neither the rate of the hystolytical processes in the caterpillars nor the rate of development in the chrysalids were in our experiments influenced by the thyroid added to the food. In other words, this substance did not elicit any changes in the course of metamorphosis of the examined insects.

From the theoretical point of view this result requires however the following remarks. It has often been emphasized that the influence of glands of internal secretion may be not only of various intensity, but even essentially different in relation to the developmental or growth stage of the animals experimented

TABLE I.

DATA CONCERNING THE TERM OF PUPATION OF THE CATERPILLARS OF *Lymantria dispar* L., IN DAYS.

The term of pupation calculated from the day of the appearance of the first pupa in the whole material, *i.e.* from June 24.

n, number of specimens; σ, standard deviation; $A \pm E_A$, average term of pupation with its probable error; $v \pm E_v$, coëfficient of variability of this term together with its probable error; Diff. $\pm E_{\text{Diff.}}$, difference together with its probable error; Diff./$E_{\text{Diff.}}$, ratio of the difference to its probable error.

Sex.	Number Corresponding to Each Experiment.	Material of Caterpillars.	n.	$\pm\sigma$.	$A \pm E_A$.	Difference between b and a in Averages.		$v \pm E_v$.	Difference between b and a in Coëfficients of Variability.	
						Diff. $\pm E_{\text{Diff.}}$	Diff./$E_{\text{Diff.}}$		Diff. $\pm E_{\text{Diff.}}$	Diff./$E_{\text{Diff.}}$
Males	1	(*a*) "Thyroid"	59	3.17	8.98 ±0.28	−0.36 ±0.36	1.0	35.3 ±2.45	−9.9 ±3.11	3.2
		(*b*) Control.....	45	2.19	8.62 ±0.22			25.4 ±1.92		
	2	(*a*) "Thyroid"	68	2.13	6.21 ±0.17	+0.86 ±0.23	3.7	34.3 ±2.21	−9.3 ±2.76	3.4
		(*b*) Control.....	58	1.77	7.07 ±0.16			25.0 ±1.66		
	3	(*a*) "Thyroid"	50	2.57	10.32 ±0.25	+0.24 ±0.37	0.6	24.9 ±1.78	−0.9 ±2.63	0.3
		(*b*) Control.....	39	2.53	10.56 ±0.27			24.0 ±1.94		
	4	(*a*) "Thyroid"	57	2.61	6.81 ±0.23	−1.09 ±0.37	2.9	38.3 ±2.75	+2.3 ±4.98	0.5
		(*b*) Control.....	29	2.32	5.72 ±0.29			40.6 ±4.15		
Females	1	(*a*) "Thyroid"	55	3.58	13.20 ±0.33	+0.37 ±0.47	0.8	27.1 ±1.87	+2.0 ±2.65	0.8
		(*b*) Control.....	64	3.95	13.57 ±0.33			29.1 ±1.88		
	2	(*a*) "Thyroid"	48	2.29	11.96 ±0.22	−0.81 ±0.32	2.5	19.1 ±1.36	+4.7 ±2.07	2.3
		(*b*) Control.....	59	2.65	11.15 ±0.23			23.8 ±1.56		
	3	(*a*) "Thyroid"	37	3.46	14.92 ±0.38	−0.73 ±0.49	1.5	23.2 ±1.91	+0.4 ±2.52	0.2
		(*b*) Control.....	52	3.35	14.19 ±0.31			23.6 ±1.65		
	4	(*a*) "Thyroid"	48	4.41	11.96 ±0.43	−1.72 ±0.57	3.0	36.9 ±2.87	+2.1 ±4.15	0.5
		(*b*) Control.....	50	3.99	10.24 ±0.38			39.0 ±3.00		

TABLE II.

DATA CONCERNING THE DURATION OF PUPAL PERIOD OF *Lymantria dispar* L., IN DAYS.

n, number of specimens; σ, standard deviation; $A \pm E_A$, average duration together with its probable error; $v \pm E_v$, coëfficient of variability of the duration together with its probable error; Diff. $\pm E_{\text{Diff.}}$, difference together with its probable error; Diff. $/E_{\text{Diff.}}$, ratio of the difference to its probable error.

Sex.	Number Corresponding to Each Experiment.	Material of Chrysalids.	n.	$\pm\sigma$.	$A \pm E_A$.	Difference between b and a in Averages.		$v \pm E_v$.	Difference between b and a in Coëfficients of Variability.	
						Diff. $\pm E_{\text{Diff.}}$	Diff. $/E_{\text{Diff.}}$		Diff. $\pm E_{\text{Diff.}}$	Diff. $/E_{\text{Diff.}}$
Males	1	(*a*) "Thyroid"	57	1.37	17.18 ± 0.12	+0.15 ± 0.17	0.9	7.97 ± 0.50	−1.10 ± 0.71	1.5
		(*b*) Control.....	42	1.19	17.33 ± 0.12			6.87 ± 0.51		
	2	(*a*) "Thyroid"	65	1.21	17.15 ± 0.08	+0.10 ± 0.11	0.9	7.06 ± 0.42	−1.96 ± 0.53	3.7
		(*b*) Control.....	57	0.88	17.25 ± 0.08			5.10 ± 0.32		
	3	(*a*) "Thyroid"	46	1.12	18.72 ± 0.11	+0.28 ± 0.19	1.5	5.98 ± 0.42	+1.55 ± 0.74	2.1
		(*b*) Control.....	35	1.43	19.00 ± 0.16			7.53 ± 0.61		
	4	(*a*) "Thyroid"	50	1.18	16.58 ± 0.11	+0.01 ± 0.19	0.05	7.12 ± 0.48	−0.31 ± 0.78	0.4
		(*b*) Control.....	27	1.13	16.59 ± 0.15			6.81 ± 0.62		
Females	1	(*a*) "Thyroid"	53	1.54	15.87 ± 0.14	+0.16 ± 0.18	0.9	9.70 ± 0.64	−0.65 ± 0.84	0.8
		(*b*) Control.....	63	1.45	16.03 ± 0.12			9.05 ± 0.54		
	2	(*a*) "Thyroid"	47	1.17	15.89 ± 0.12	−0.09 ± 0.17	0.5	7.36 ± 0.51	+1.63 ± 0.76	2.1
		(*b*) Control.....	59	1.42	15.80 ± 0.12			8.99 ± 0.56		
	3	(*a*) "Thyroid"	34	1.31	16.65 ± 0.15	+0.45 ± 0.18	2.5	7.87 ± 0.64	−1.55 ± 0.77	2.0
		(*b*) Control.....	50	1.08	17.10 ± 0.10			6.32 ± 0.43		
	4	(*a*) "Thyroid . . "...	48	1.71	15.58 ± 0.17	−0.19 ± 0.23	0.8	10.98 ± 0.76	−0.91 ± 1.03	0.9
		(*b*) Control.....	49	1.55	15.39 ± 0.15			10.07 ± 0.69		

upon (cf., *e.g.*, the manual of Biedl, '22, as well as the papers of Romeis, '14–'15, '16 and '18, Gedroyć, '23, Deutsch, '23, and others). Consequently the absence of any considerable and constant differences in the processes of metamorphosis between my control animals and those fed on thyroid, does not solve our problem. We ought to take into consideration, that owing to individual fluctuations on the starting day of the thyroid feeding, *i.e.* on June 5th, the larvæ experimented upon without any doubt differed from one another considerably both in regard to weight and most probably also to stage of development, although having hatched during one and the same night. It therefore was a priori not known whether the average terms of metamorphosis remained unchanged in spite of the different influence exerted by thyroid on separate specimens. For instance, if the positive and the negative influence exerted by thyroid feeding on separate caterpillars were of identical intensity, the average duration of the larval as well as of the pupal stage would remain unchanged. The so to speak latent influence of thyroid feeding ought to be noticeable here only by the variation of the terms of metamorphosis, as the range of individual fluctuations of the terms of pupation and emergence of adult moths would in such case undergo in the experimental materials a distinct widening. As a matter of fact in my experiments no remarkable changes in the coefficients of variability of these terms of the mentioned variation may be seen (cf. Tables I. and II.). The differences of the appertaining coefficients between control and thyroid materials have opposite signs, in one case being positive, in the remaining negative. The ratio of the difference between the coefficients to its probable error was only in few cases larger than 3, as a rule attaining from 0.2 to 2.3 only. It follows therefrom that these biometrical tests show no distinct influence of thyroid feeding on the rate of metamorphosis of the insects experimented.

The above results require a certain supplement in still another direction. Viz: the question arises whether in these experiments the lack of influence of thyroid on metamorphosis is caused by too small quantities of the substance added, or unsuitable age of the animals at the beginning of the experiments. Decisive

answers to this question have been attained, according to my view, by weighing fresh pupæ. It appeared that the growth of the caterpillars (the term of which was determined by the weight of fresh chrysalids) underwent in all cases, without any exception, a considerable retardation under the influence of thyroid feeding (cf. Table III.). The differences between the control and the experimental material are here always positive, in 3 cases being biometrically very significant. The thyroid had therefore in this respect an undoubtedly negative influence on the processes of larval growth, *i.e.*, the dosing of thyroid as well as the age of the animals have been well chosen to demonstrate the supposed influence of this substance. It is very characteristic that the variability of weight of the "thyroid" caterpillars does not undergo any essential and corresponding changes (cf. Table III.). This points to the fact that the negative influence exerted here by thyroid feeding on growth of caterpillars had the same qualitative and quantitative effect for all animals.

The influence of organic or even inorganic compounds of iodine on amphibian metamorphosis is much discussed in recent years and the problem contains certain discrepancies (cf., *e.g.*, the papers of Romeis, '18, Allen, '19, Hirschler, '18–'19 and '22, Huxley, '22, Huxley and Hogben, '22, Uhlenhuth, '22, and others). Gedroyć ('23) studied the problem in respect to insects, viz. on *Deilephila euphorbiæ* L. In two experiments each containing 5 "iodine" caterpillars, the author observed an acceleration of the pupation of the experimental specimens relatively to the 15 control caterpillars. On the contrary, in the third analogous experiment in which also 5 animals, but 2 or 4 days younger than the larvæ of the preceding series, were used, the larval period underwent in the "iodine" material even a certain prolongation. Hence the inference of Gedroyć as to the decisive influence of age and "state of growth" of the animals exerted on the direction of the action of iodine.

The material of Gedroyć being scanty I have studied anew the problem of the influence of iodine on insect metamorphosis. My experiments were performed on caterpillars of *Pieris brassicæ* L. Larvæ hatched between August 2d and 3d were used in all 4 experiments. Cabbage leaves abundantly sprayed with Lugol's

TABLE III.

DATA CONCERNING THE WEIGHT OF CHRYSALIDS OF *Lymantria dispar* L., IN MG.

n, number of specimens; σ, standard deviation; $A \pm E_A$, average weight together with its probable error; $v \pm E_v$, coëfficient of variability of the weight together with its probable error; Diff. $\pm E_{Diff.}$, difference together with its probable error; Diff./$E_{Diff.}$, ratio of the difference to its probable error.

Sex.	Number Corresponding to Each Experiment.	Material of Chrysalids.	*n*.	$\pm\sigma$.	$A \pm E_A$.	Difference between *b* and *a* in Averages.		$v \pm E_v$.	Difference between *b* and *a* in Coëfficients of Variability.	
						Diff. $\pm E_{Diff.}$	Diff./$E_{Diff.}$		Diff. $\pm E_{Diff.}$	Diff./$E_{Diff.}$
Males	1	(*a*) "Thyroid"	59	70.6	367.0± 6.2	+14.1±10.7	1.3	19.2±1.24	+3.6±2.10	1.7
		(*b*) Control.....	45	86.8	381.1± 8.7			22.8±1.70		
	2	(*a*) "Thyroid"	68	69.5	408.8± 5.7	+18.8± 9.4	2.0	17.0±1.01	+2.7±1.63	1.7
		(*b*) Control.....	58	84.3	427.6± 7.5			19.7±1.28		
	3	(*a*) "Thyroid"	50	89.9	428.0± 8.6	+11.7±12.4	0.9	21.0±1.48	−2.3±2.09	1.1
		(*b*) Control.....	39	82.2	439.7± 8.9			18.7±1.48		
	4	(*a*) "Thyroid"	57	79.6	430.7± 7.1	+60.7±13.5	4.5	18.5±1.21	+0.2±2.09	0.1
		(*b*) Control.....	29	92.1	491.4±11.5			18.7±1.71		
Females	1	(*a*) "Thyroid"	55	248.4	1078.2±22.6	+90.6±32.5	2.8	23.0±1.56	+0.7±2.16	0.3
		(*b*) Control.....	64	277.4	1168.8±23.4			23.7±1.49		
	2	(*a*) "Thyroid"	48	299.6	1383.4±29.2	+92.8±42.4	2.2	21.7±1.56	+2.0±2.20	0.9
		(*b*) Control.....	59	350.5	1476.2±30.8			23 7±1.55		
	3	(*a*) "Thyroid"	37	307.8	1175.6±34.1	+216.6±46.6	4.6	26.8±2.25	−2.4±2.83	0.8
		(*b*) Control.....	52	340.3	1392.2±31.8			24.4±1.71		
	4	(*a*) "Thyroid"	48	237.7	1187.6±23.1	+440.4±41.4	10.6	20.0±1.43	+2.1±2.12	1.0
		(*b*) Control.....	50	359.3	1628.0±34.3			22.1±1.56		

solution (6 g. IK, 4 g. I in 100 g. H_2O) were daily administered to the caterpillars of experiments 1 and 2 from August 16th, and in those of experiments 3 and 4 from August 22d. Leaves designed for control animals were similarly changed daily and sprayed with water. All animals were kept under glass vessels. The administered leaves were taken by the "iodine" caterpillars as well as by those of control materials. Data referring to the pupation *term* of these caterpillars as well as to the average *weight* of the resulting chrysalids are recorded in Table IV.

TABLE IV.

DATA CONCERNING THE TERM OF PUPATION AND THE WEIGHT OF PUPÆ FROM "IODINE" CATERPILLARS OF *Pieris brassicæ* L.

The terms of pupation calculated from the beginning of iodine feeding.

Number Corresponding to Each Experiment.	Material of Specimens.	Number of Specimens.	The Average Term of Pupation, in Days.	Difference between *b* and *a*.	The Average Weight of Chrysalids, in mg.	Difference between *b* and *a*.
1	(*a*) "Iodine" . .	12	12.2	− 2.4	294.4	+ 76.5
	(*b*) Control. . . .	23	9.8		370.9	
2	(*a*) "Iodine" . .	11	12.1	+ 0.9	364.1	+ 10.9
	(*b*) Control. . . .	15	13.0		375.0	
3	(*a*) "Iodine" . .	8	8.5	+ 2.5	311.5	+ 67.1
	(*b*) Control. . . .	10	11.0		378.6	
4	(*a*) "Iodine" . .	6	12.0	− 2.7	251.7	+ 59.6
	(*b*) Control. . . .	6	9.3		311.3	

It is evident that neither in the case when iodine feeding was begun earlier (first and second experiment) nor in that started almost a week later (third and fourth experiment) the obtained results were concordant. The sign of the difference between the terms in control animals and those for the experimental materials was different in both cases which points to the lack of any influence of iodine feeding. On the other hand the average weight of the "iodine" chrysalids was here also always smaller than that of the control pupæ. The total of the recorded chrysalids amounted here to 37 "iodine" and 54 control specimens. My material was therefore larger than that of Gedroyć. It was nevertheless not large enough to consider the results obtained as decisive, all the more as the insects were not

segregated in respect to sex. Further and more detailed research ought to be undertaken in this respect.

It ought to be remarked, that notwithstanding such distinct influence of both thyroid and Lugols' solution on growth of caterpillars, mortality in the animals of the experimental materials was in general not larger than in those of the control. The average mortality in male "thyroid" larvæ attains ca. 6 per cent., in females 5 per cent., in the control materials 4 per cent. in males and 7 per cent. in females. Analogous data for "thyroid" chrysalids was 7 and 3 per cent., in control pupæ 6 and 2 per cent. The larvæ of *Pieris brassicæ* reared as a rule very badly in the extremely damp air under glass, but also here no specially negative influence of the experiment could be observed. Viz. the average mortality in the "iodine" caterpillars was here approximately 43 per cent., in the control material 38 per cent.

TABLE V.

THE COËFFICIENTS OF CORRELATION BETWEEN THE WEIGHT OF CHRYSALIDS OF *Lymantria dispar* L. AND THE LENGTH OF THE LARVAL PERIOD.

$r \pm E_r$, coëfficient of correlation together with its probable error; r/E_r, ratio of the coëfficient to its probable error.

Material of Specimens.	Number Corresponding to Each Experiment.	Males.		Females.	
		$r \pm E_r$.	r/E_r.	$r \pm E_r$.	r/E_r.
"Thyroid"	1	− .425 ± .072	5.9	+ .095 ± .090	1.1
	2	− .161 ± .080	2.0	− .408 ± .081	5.0
	3	− .506 ± .071	7.1	− .207 ± .106	2.0
	4	− .652 ± .051	12.8	− .251 ± .091	2.8
Control.	1	− .476 ± .078	6.1	+ .033 ± .084	0.4
	2	− .278 ± .082	3.4	+ .460 ± .069	6.7
	3	− .415 ± .089	4.7	− .130 ± .092	1.4
	4	− .606 ± .079	7.7	+ .279 ± .088	3.2

After the present experiments were finished an abstract appeared [1] referring to a recently published preliminary note of Terao and Wakamori ('24) inaccessible to me. The authors report that the metamorphosis of the caterpillars of *Bombyx*

[1] *Berichte ü. d. ges. Pysiol. u. exp. Pharmak.*, Vol. 28, p. 119, November 12, 1924.

mori L. fed on mulberry leaves sprayed with thyroid extract is normal and that the "thyroid" animals are smaller than the controls. The accelerating influence of the thyroid food was however noticed in the second generation of the insects under examination. We see that the results of my "thyroid" experiments are in complete agreement with those performed by Terao and Wakamori on the first generation of the animals. Unfortunately I have been unable to examine the second generation of my moths.

I will not discuss here the relation of the lack of any influence of thyroid on the rate of metamorphosis of the insects experimented upon to the problem of the physiology of metamorphosis in these animals. As I have emphasized elsewhere (Kopeć, '23), only positive results of the experiments on the influence of glands of internal secretion may be considered as decisive in this respect and only to a certain degree. It is not impossible that the negative influence exerted in my experiments on the weight of caterpillars by thyroid feeding is not due to specific checking of growth faculties, but rather to certain changes elicited in their digestion by a totally foreign substance. Such caution is the more advisable, as we have already certain data in the literature, which point to positive effect exerted on "lower" animals by substances of glands of internal secretion from vertebrates. Nowikoff ('08) remarks an increase of the rate of multiplication of infusorians reared in aqueous extracts of the thyroid gland. Hankó ('12) observes a positive influence of extracts of hypophysis on the rate of moult, regeneration and growth in *Asellus aquaticus*. The thyroid gland has, according to Wulzen ('16) a positive influence on the rate of division and growth of *Planaria maculata*. From the experiments of van Herwerden ('23) we see that the cortical substance of adrenals of the ox has an accelerating influence on the multiplication of *Daphnia pulex* as well as on growth of this crustacean and of young specimens of *Limnæa stagnalis*. I believe however that only a larger experimental material may constitute a base for general discussion on the influence of glands of internal secretion of vertebrates on processes taking place in invertebrates.

The opinions of separate authors as to the relation of growth

to metamorphosis are not as yet in agreement. While for certain authors metamorphosis is a function of growth—for others there is no essential connection between the two processes. My experiments furnish a new support for the latter opinion, if of course we shall agree to draw inferences as to growth from the weight of the animals. We see that in "thyroid" caterpillars notwithstanding a considerably decreased weight, the rate of metamorphosis remains totally unchanged. If metamorphosis were a function of growth, the correlation between the weight of the pupæ and the duration of larval life would be, at least in normal conditions, always negative. It is therefore very characteristic that both in the "thyroid" and in the normal material the coefficients of correlation between the weight of the pupæ (*i.e.*, the measure of growth) and the duration of the larval period were, it is true, in general rather large, but not always negative (cf. Table V.). According to my opinion we may infer therefrom that between larger growth and quicker metamorphosis of caterpillars there is only the relation of contemporariness, perhaps dependent on a totally separate, unknown factor (or factors). We have not to do here with real, functional correlation between growth and metamorphosis which could not be abolished even in normal conditions.

In conclusion I want to remark that the results of my present research are by no means contradictory to my former views on the decisive rôle of the brain for insect metamorphosis, during normal development (Kopeć, '22) as well as during starvation (Kopeć, '24). The substance of the thyroid gland from vertebrates may have no relation to the substance (or substances) the existence of which ought, according to my opinion, to be considered as indispensable for the metamorphosis of these animals. I refer their presence in the insect to the presence of brain. The lack of metamorphosis in the caterpillars of *Lymantria dispar* which have been deprived of brain the seventh day after their last moult (Kopeć, '22) is sufficient evidence in this connection. The only matter for discussion is whether this influence occurs through the interaction of nerves, or, according to my supposition (not assertion), by means of internal secretion. In recent times Gedroyć ('23) opposed very decisively my

interpretation of the hormonal rôle of the brain during insect metamorphosis. Namely when grafting brains or other parts of the nervous system on normal caterpillars of *Deilephila euphorbiæ* this author did not observe any acceleration of metamorphosis. I cannot, however, consider as decisive experiments like those of Gedroyć. in which the influence of grafted parts of the nervous system are studied, the analogical parts of the host not being removed. The introduced nervous tissue is present here in excess and it *might* at most, but it *need* not elicit accelerated metamorphosis, *i.e.*, increase the effect of the function which brain probably has when present in the organism in normal quantities.

I want to explain here a certain seeming contradiction between the results of my former experiments, which has been justly pointed to by the mentioned author. Gedroyć emphasizes that from Table I. of my paper on the rôle of the brain in the metamorphosis of insects (cf. Kopeć, '22) it follows that the control caterpillars which fasted since the seventh day after their last moult, and the head of which has been injured, the brain being not removed undergo pupation simultaneously with the caterpillars which have not been operated upon at all, and which were not deprived of food. On the contrary from my later paper on the influence of starvation on the development of insects (cf. Kopeć, '24) it follows that caterpillars which have been subjected to inanition since the seventh day after their last moult undergo pupation later than the unstarved controls. This discrepancy is however not essential for the caterpillars subjected in either case to starvation the seventh day after their last moult were not physiologically identical. In the second case (Kopeć, '24) they were normally fasting animals, in the first (Kopeć, '22) injured similarly to those the brain of which has been removed. Owing to the severe injury, connected with loss of blood, these caterpillars were not able to take food and were therefore subjected indirectly to starvation. I have twice just emphasized in connection with other experiments (Kopeć, '08 and '11) that serious operations on caterpillars may have an accelerating influence on the succeeding moult, which in the above mentioned case corresponded to pupation. This acceler-

ating influence on metamorphosis may thus have annulled in the case from my paper of 1922 the hindering influence of starvation. In other words, probably owing to mutual counteraction of the two opposite influences the normal term of pupation was maintained here.

From the foregoing inquiry the following summary may be given:

1. The administration of thyroid to caterpillars of *Lymantria dispar* L. did not cause any distinct changes in the duration of the larval nor of the pupal period. The only effect was a distinct diminution of the weight of the chrysalids.

2. Neither the variability of the pupation term, nor that of the emergence of the moths, nor of the pupal weight underwent any regular or essential changes as compared with corresponding items of the control materials.

3. Lugols' solution (IK + I) had no distinct influence on the rate of pupation of the caterpillars of *Pieris brassicæ* L. and its only consequence was the negative changes of pupal weight. In respect to the scanty material these experiments are however not considered as decisive.

4. Negative correlation between the growth of caterpillars of *Lymantria dispar* L. (the growth being expressed as weight of the pupæ) and the duration of larval life cannot always be observed. This fact as well as the diminution of growth in spite of the unaltered rate of metamorphosis in the "thyroid" experiments point to phenomena of concommitance but not of functional correlation normally existing between metamorphosis and growth of insects.

5. The mortality of the "thyroid" as well as of the "iodine" specimens was not larger than in the control materials.

6. The hypothesis formerly uttered by the author as to the hormonal influence exerted by the brain on insect metamorphosis is by no means abolished by the negative results of the present experiments. Neither is this hypothesis cancelled by the experiments and considerations of Gedroyć.

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SINGLE AND DOUBLE RINGS AT THE REDUCTION DIVISION IN UVULARIA.

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It has not, apparently, been yet ascertained in what points the reduction division in flowering plants differs from the corresponding division in animals, such as the insects (see Tischler, 1922). The absence of a centrosome, indeed, has been noted in many flowering plants; and it has also been presumed that the division of the homologous chromosomes into chromatids is not visible before the early anaphase. But whether the complicated changes described, for instance, by Janssens (1924) as occurring in insect chromosomes and summarized by Wilson (1925), are paralleled in flowering plants, is, it appears, not known (though Chodat, 1925, considers that *Allium* offers a parallel). The following is a small contribution to the determination of the likenesses and differences of the maturation divisions of flowering plants and those of the best known animals, say, the Orthoptera.

As compared with most animals, flowering plants may differ in showing a periodicity in the occurrence of the maturation divisions corresponding with the alternations of day and night. Certain stages of *Uvularia*, for example, apparently usually came late at night and hence were rarely obtained under normal environment in the daytime. However, cold checks or stops the process; and if preparations are made at intervals after cold has occured in the night, various less common stages can often be procured in the daytime. The first and second metaphases of *Uvularia* have been readily obtainable under the usual environment; but the stages just previous to the first metaphase, and those showing the separation of the bivalents into their component chromosomes, or chromatids, were not often seen when preparations were made at 9 or 10 o'clock in the morning. The preparations were mostly procured after the young buds had been somewhat chilled by the temporary lowering of the night

temperature in February and March, when the greenhouse fires were banked.

The preparations of young microspores and pollen-mother-cells were fixed and stained in iron-acetocarmine (Belling, 1921). A water-immersion objective was used to examine the chromosomes. It may be noted that an apochromatic water-immersion of 1.25 aperture will allow a true condenser aperture of 1.2, if the source of light is diaphragmed to fit the field of view. The working aperture of the combination is about 1.2. On the other hand, the working aperture of the 1.4 N.A. apochromatic oil-immersion, used with a dry Abbe uncorrected condenser on objects in iron-acetocarmine, is only 0.9 or less. (Compare Belling, 1923.)

Three hypotheses have been made as to the rings seen at the metaphase in certain Liliaceæ and other genera of flowering plants. (1) That these rings result from the mere twisting of the constituent chromosomes of the bivalents around one another, and that these become untwisted and longitudinally split at the anaphase. This hypothesis is, it would seem, negatived by the fact that, with the best visibility in the microscope, it is usually impossible to say which chromosome is above and which below at the junctions. (2) That the rings are caused by alternate openings between the homologues and between their constituent chromatids, so that adjacent rings are always at right angles. This hypothesis does not appear to agree well with the state of affairs in the trivalents of the triploid hyacinths, where 3 chromosomes are concerned (Belling, 1925*a*). (3) That between each two adjacent rings there is a chiasma in Janssens' sense (or a similar interlacing of strands) where segmental exchange may have taken place, by the fracture and subsequent reciprocal junction of two of the four strands. This assumption seems most suitable for a working hypothesis, since crossing-over (and hence probably segmental interchange) has been shown to occur in those flowering plants which have been investigated *ad hoc*.

However, in the present paper only the following points are regarded.

1. Whether in horizontal rings or V's one chromatid passes up and one down from each lateral half of the ring or V.

2. Whether these chromatids in separating show signs of inter-

lacing at the junction, so that a ring or V gradually diminishes in size as its chromatids are pulled into the loop by the spindle fibers.

3. Whether, in vertical rings or V's, the process is the same as the above; or whether the upper and lower halves of the rings or V's separate as wholes.

4. What different configurations are shown by the same homologous chromosomes in different cells.

Bivalent I.

Chromosome I., in the pollen grain (Fig. 1), is seen to be formed of two slightly unequal segments, and is usually bent; the spindle

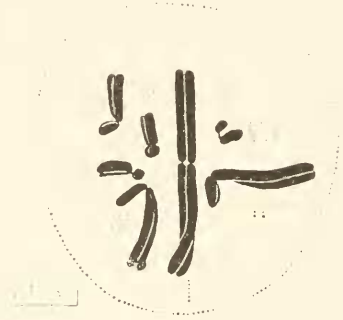

Fig. 1. This shows the metaphase at the first division in the pollen-grain. (Like all the other figures it was made from preparations of *Uvularia grandiflora*.) This pollen-grain was selected from a large number as showing the chromosomes well spaced. The longitudinal divisions and the constrictions are evident. It can be seen that the two segments of chromosome I. are of unequal length. Chromosome III. has apparently a shorter long segment and a longer short segment than chromosome II. The small segment of chromosome V. is closely attached to the large segment, while in chromosome VI., the two segments are separated by a thread.

This, and the subsequent figures were drawn with the camera from iron-aceto-carmine preparations, the light being screened by Wratten yellow-green filter No. 56. A Leitz' achromatic aplanatic condenser, corrected for use as a water-immersion, was used to give a large cone of light. Zeiss' apochromatic water-immersion 70 was employed in all cases.

fiber being, of course, attached at the constriction, where the two segments are connected by a fine thread. Fig. 2 of the paper on the origin of chromosomal mutations (Belling, 1925*b*) shows bivalent I. in the late prophase in the form of two medium-sized

rings with a long V, the plane of the V being at right angles to that of the adjacent ring. At the metaphase, as shown in Fig. 2 of the present paper, one large ring and V may be present. The

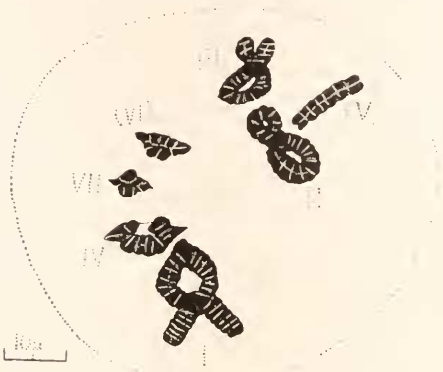

FIG. 2. First metaphase (to anaphase) in a pollen-mother-cell, from a plant forced in the greenhouse, in March, 1923. This cell was selected as showing no overlapping bivalents. In bivalents I., II. and III., the spindle fibers would probably be attached where the constituent chromosomes seem to cross.

division into chromatids cannot be seen in face view, but only when the rings or V's are presented edgeways. In the next stage, which is not figured here, a small loop arises from the

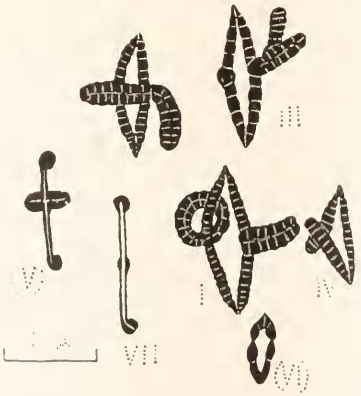

FIG. 3. First metaphase to anaphase in a pollen-mother-cell. Drawn in March, 1924; after a spell of cold weather. The drawing surface was shifted after outlining each bivalent, as was done also in Figs. 4 and 5. Bivalent VI. has the more or less exceptional configuration noted in the text in bivalent IV. Bivalent VII. is the most advanced in separation.

apposed points of constriction of the homologues, and is pulled out by the spindle fibers towards each pole, pulling the chromatids out of the rings or V's as it apparently increases in size at the expense of these rings or V's. In Fig. 3, bivalent I. consists of a large loop with two medium-sized rings; one of which is seen edgeways. This ring shows the division into chromatids. This bivalent perhaps started as two rings. In Fig. 5, lower line,

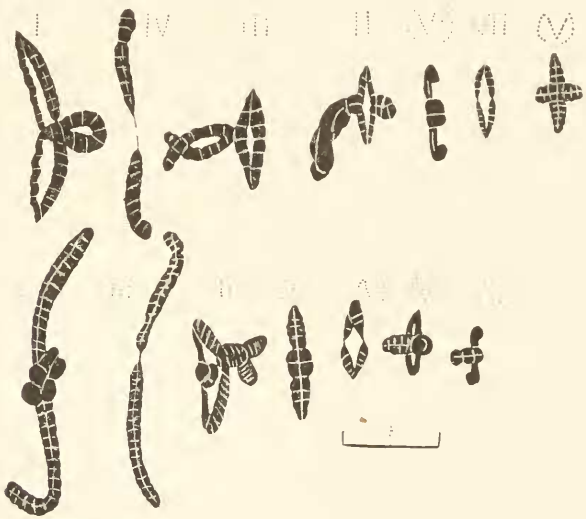

FIG. 4. Bivalents from two more selected cells showing the metaphase to anaphase, as in Fig. 3. Two bivalents, VI. and VII., are of the exceptional form.

bivalent I. consists of a diminishing ring and a V, with a large loop between them, formed probably at their expense. Bivalent I., in the upper line of this figure, is apparently similar but the ring is somewhat bent. In Fig. 3, of the paper on the origin of mutations, bivalent I. has the form of a large loop with two small rings, probably remnants of original large rings. In Fig. 4 of the present paper, upper line, bivalent I. has a ring on one side of the loop, while on the other side the ring may perhaps have been vertical, and the two halves have separated vertically. In the lower line of this figure, the halves of bivalent I. have separated at one end, but the last remnant of the V remains at the center. Finally, in Fig. 6, the 4 chromatids of bivalent I. are

clearly visible; and it may be that there had been a horizontal ring on the right, and a vertical ring on the left.

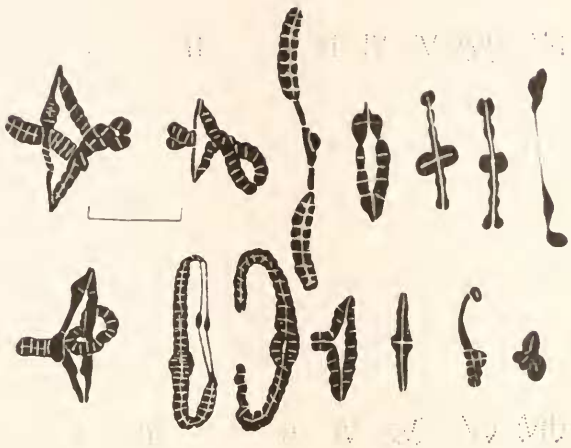

FIG. 5. Two more selected metaphase to anaphase groups. The last two bivalents in the lower line seem more or less malformed.

BIVALENTS II AND III.

Chromosomes II. and III. (Fig. 1) doubtless differ slightly in size, but are similar in behavior, and may be considered together. They are long J chromosomes, with a large and a small segment close together. At the late prophase (Fig. 2, Belling, 1925*b*) these two bivalents may have each the form of a large horizontal ring with a small V at one end, and a vertical portion consisting of the two small segments at the other end. At the metaphase (in Fig. 2, of the present paper), one of these bivalents forms a ring with a small V, and the other a large and small ring in different planes. Fig. 4 of the paper on the origin of mutations shows bivalent II. forming a horizontal ring with a small vertical piece consisting of the two small segments, and with a small loop arising from the constrictions. In the upper line of Fig. 4 of the present paper, this loop has increased in size in bivalent II. In Fig. 4, lower line, and Fig. 5, upper line, bivalent II. shows part of the small segments still in the equatorial plane, as well as a ring (or ring and V) on the other side. But in bivalents II. and III. of Fig. 3, the small segments have been quite drawn into the loop, and only the diminishing V is left; or in bivalent III. of the

upper line of Fig. 4, the ring and V. In Fig. 5, lower line, both short and long segments have been drawn into the loop. Fig. 6 is especially instructive, for bivalent II. probably had consisted of

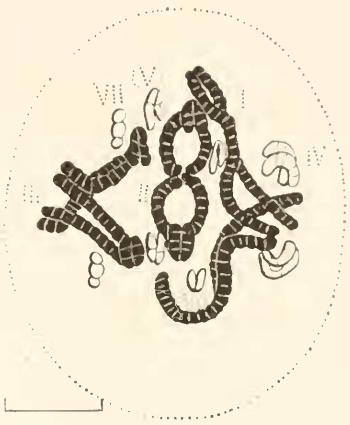

FIG. 6. A rare stage of the pollen-mother-cell at the early anaphase, showing the final separation of bivalents I., II. and III. The arrangement of the chromatids of bivalent II. must apparently be due to the horizontal splitting of a horizontal ring. The four smaller bivalents are, as usual, ahead of the larger ones in completing the separation of their constituents.

a large horizontal ring with a vertical piece formed of the two short segments. This is seen to have separated by the horizontal splitting of the ring into its constituent chromatids, the small segments having first separated. In bivalent III. of Fig. 6, a horizontal V has, it seems, separated into chromatids in the same way.

BIVALENT IV.

Chromosome IV., as shown in Fig. 1, consists of a small J, in which the two segments are close together. It is distinctly larger than either V. or VI. In Fig. 2 of the paper on the origin of mutations, bivalent IV. is formed of a horizontal V, with a vertical piece consisting of the two short segments. In Fig. 2 of the present paper, this bivalent, which apparently started as a ring, is already in the early anaphase (for the small bivalents usually separate before the large ones). In Fig. 3, bivalent IV., which possibly started as a ring and V, still shows a small V at the left of the loop. A later stage is shown in the lower line of Fig. 5. In the top of Fig. 4, the two halves of bivalent IV. have separated

without splitting lengthways. The constriction between the short and long segments is visible. The upper line of Fig. 5 shows a different configuration for bivalent IV., in which the two sides of the loop are equal. This is intelligible, first if we suppose the point of attachment of the spindle fiber and the constriction to have been shifted to the center of both chromosomes, which seems unlikely. Or we may presume that a horizontal V has separated into its chromatids, somewhat as bivalent III. of Fig. 6.

Bivalents V. and VI.

Chromosomes V. and VI., since they differ only in VI. having its small segment attached by a long filament, cannot be told apart in the maturation divisions, and must therefore, be considered together. In Fig. 2 of the paper on the origin of mutations, bivalents V. and VI. have the form of V's or truncated A's. In Fig. 3 of the present paper, in the configuration marked (V.), the two small segments have separated, and the two large segments are still horizontal and show the split between the chromatids. Similar forms may be seen in the top line of Fig. 5. The bivalent marked (VI.) in Fig. 3, however, like the one in the bottom line of Fig. 4, is presumed to have come from the separation of the chromatids of a horizontal V.

Bivalent VII.

Finally bivalent VII. resembles V. and VI. in its configurations, but is recognizably smaller.

To sum up: (1) There is, apparently, fusion at the junctions of the two constituents of each bivalent. (2) Horizontal rings and V's divide into their constituent chromatids along a horizontal plane, the line of division being distinct at the metaphase. (3) Vertical V's and rings have not been observed in the act of separating; but they probably separate into upper and lower halves. (4) The rings and V's apparently diminish as the loop between them is pulled out by the spindle fibers. This would presuppose crossing or interlacing of the chromatids.

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BIOLOGICAL BULLETIN

ON THE PHOTOGENIC ORGAN OF THE KNIGHT-FISH (*MONOCENTRIS JAPONICUS* (HOUTTUYN)).

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It was Stead (1906, p. 89) who first described the luminescence of the knight-fish (*Monocentris gloria-maris*). "On each side of the head near the mouth are peculiar luminous discs, which are probably of service to the fish in assisting it to obtain its food." Unfortunately this interesting observation has escaped attention up to this date.

Monocentris japonicus, another well-known species of the knight-fish, is a rather common animal along the coast of Japan. This fish also has the same property of light production as the Australian form. Light is produced by a pair of glands situated just under the lower jaw of the fish. Their function and structure were described by Yoshizawa (1916, p. 411) in Dobutsu-Gaku-Zashi, a Japanese zoölogical periodical. His brief description was illustrated with figures, but several points remain unexplained. According to Yoshizawa the photogenic organs of *Monocentris* have the construction of photospheres, composed of large glandular cells with nuclei of a considerable size, but without accessory structures such as the reflector. He seems not to have been aware of the truly glandular nature of the organs.

In 1917 Harvey came to Japan and made at the Misaki Biological Station some observations upon the interesting luminous shore-fish. These observations have been recorded in several of his papers. The photogenic property of *Monocentris* thus has come to be generally known as a fact.

Localization and General Structure of the Photogenic Organs.

The photogenic organs of *Monocentris* consist of two ovoid protuberances lying side by side on each side of the median thickening of the lower jaw, their longer diameter being nearly at right angles to the median plane (Fig. 1, *gl*). In a fish 12 cm. long the protuberances measure about 4 mm. in length and 3 mm. in width. Their whole surface being covered with minute dermal papillæ of dark brown color, the photogenic protuberances do not stand out clearly above the surrounding

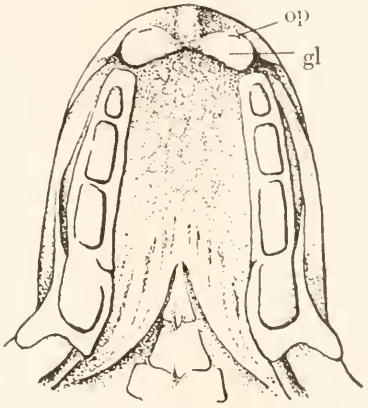

Fig. 1. Ventral view of the lower jaw of *Monocentris japonicus*. About × 2. *gl*, photogenic gland; *op*, opening of gland.

surfaces. This fact seems to be the reason why the organs in question have remained unnoticed by most ichthyologists.

The photogenic organs of *Monocentris* are glands of an excretory nature, although luminous material does not escape under normal conditions. The glandular nature of the organs can easily be demonstrated even without dissection. Each photogenic protuberance has a slit-like opening at the anterior end (Fig. 1, *op*) and when pressed under conditions of darkness a luminous fluid is observed to be forced out through this point. The opening is crescentic and its longer diameter is almost parallel to that of the protuberance.

The interior of the photogenic organs is white and spongy, containing a large quantity of minute crystals. A great number of simple tubules develop around the central tissue.

They crowd so thickly that each stands almost vertically, and they lie parallel to one another. Indeed, the tubules appear to belong to a single gland with several large central spaces (Fig. 3, pl. I.).

Each photogenic organ of *Monocentris* is, however, a compound gland made up of some nine components which unite secondarily into a functional whole. The distal part of the gland still shows the compound nature and this part consists of many narrow canals, each representing the duct of an individual acinous

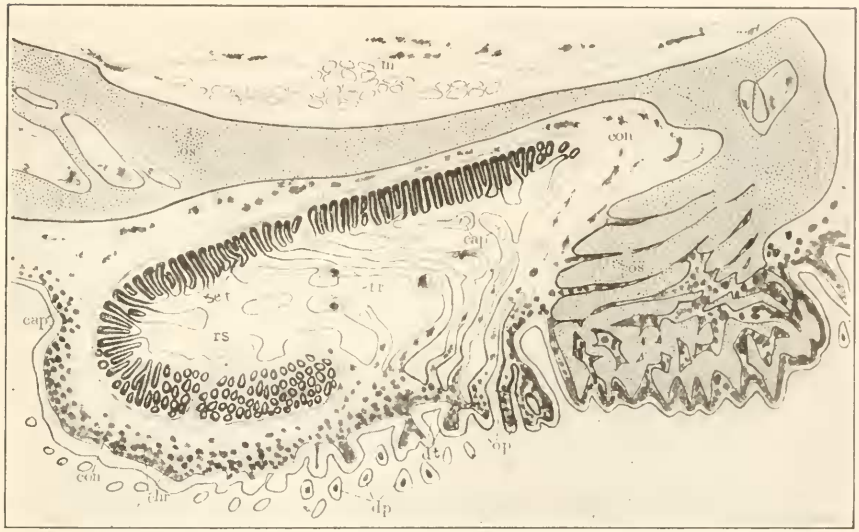

FIG. 2. Sub-mental photogenic gland of *Monocentris japonicus* in longitudinal section passing through its opening. About × 30. *cap*, capillaries; *chr*, chromatophores; *con*, connective tissue; *dp*, dermal papillæ; *dt*, emissory ducts; *m*, muscles; *rt*, reservoir; *op*, openings of gland; *os*, sub-maxillary bone; *se. t*, secretory tubules; *tr*, trabeculæ.

gland. The general constitution of the organ is represented in Fig. 2.

HISTOLOGY OF THE PHOTOGENIC ORGANS.

Under a high magnification each tubule shows an epithelial lining composed of cubical cells arranged in a single layer except at the fundus region which is the seat of a most active secretion, where the walls are considerably thickened with two or three cell layers (see Fig. 4, pl. I.). The stratified aspect of this

region is, however, not due to the direction of cutting but is the result of actual proliferation of epithelial cells. Toward the exterior at a certain distance from the fundus the stratified aspect is no more to be noticed, the cells being generally lower and the secretion less in amount. Thus, in transverse sections of the tubule, the fundus and distal region show a structural difference, the walls of the former being thick and stratified, while those of the latter are thin and simple. Naturally the difference is a gradual one. The caliber and length of the tubules are also variable, but the largest ones are generally situated at the proximal region of the gland.

A certain quantity of secretion is always found in the tubules. It is granular in composition and readily stainable with hæmatoxylin. The epithelium of the tubule is completely destroyed in the process of secretion instead of repeating its function a number of times as ordinary secretory epithelium does. Each cell of the epithelium, in this case, does not function independently but operates in connection with others along the considerable extent of the walls of the tubule. The new epithelium is replaced by the constant proliferation of cells. The process is especially active at the fundus region of the tubule, and here the process is so active that more than one layer is produced before the uppermost one is entirely destroyed. This fact gives rise naturally to the stratified aspect of the epithelium at the fundus region. The nuclei of these cells are ovoid and contain the chromatic substance in normal amount. They do not deteriorate in a degree visible to the eye as far as the cellular destruction occurs. By the time when the cell body is completely disintegrated and the nuclei are set free into the lumen of the tubule, the latter lose their internal structure, their chromatic substance being reduced in amount. Yet the nuclear membrane remains unaltered and shows, adhering to its inner surface, the last scattered traces of the chromatic substance (Fig. 5, *n'*, pl. I.).

As has been stated, the secretion is of granular constitution. Whether or not the granules are preëxistent in the cells is difficult to say. They have been described in the cells of *Anomalops* and *Photoblephalon*, two luminous shallow-water fishes in the

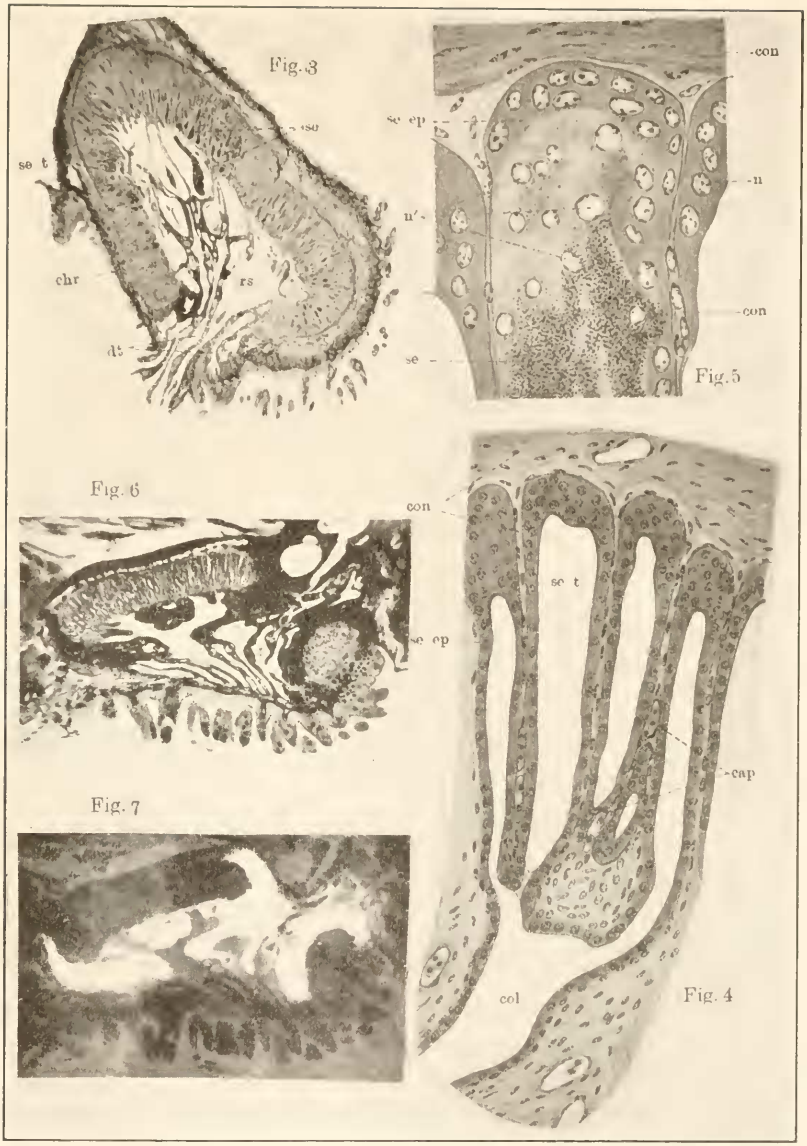

FIG. 3. Sub-mental photogenic gland of *Monocentris japonicus* in horizontal section at the level of its opening. × 16. *chr*, pigment layer; *dt*, emissory ducts; *rs*, reservoir; *se*, secretion; *se t*, secretory tubules.

FIG. 4. A portion of gland proper. × 640. *cap*, capillaries; *col*, collecting tubule; *con*, connective tissue; *se ep*, secretory epithelium; *se t*, secretory tubule.

FIG. 5. Basal part of a secretory tubule. × 1,250. *con*, connective tissue; *n*, nuclei in epithelium; *n'*, nuclei in secretion; *se*, secretion; *se. ep*, secretory epithelium.

FIG. 6. Sub-mental photogenic gland of *Monocentris japonicus* viewed by transmitted light. × 16.

FIG. 7. The same gland seen by reflected light. Crystalline deposit is represented in white.

Malayan Sea, and the photogenic organs of these fishes closely resemble those of *Monocentris* (see Steche, 1909, p. 349). No granules have, however, been detected in the cells of the latter. I am rather of the opinion that the granules are produced through the metamorphosis of the cytoplasm at the time of cellular destruction as Förster (1914) has described in *Pholas dactylus*. On the other hand, Harvey (1921, p. 43) claims the granular bodies in the tubules to be symbiotic bacteria which cause the luminescence. To this problem I shall return on another occasion.

The secretion produced in the way just considered is carried from the tubules toward the medullary part of the organ through a number of tubules of the second order, or the "collecting tubules" (Fig. 4, *col*). The latter proceed more or less parallel to one another but separated by irregular trabeculæ of connective tissue. They communicate often in their course and finally at the centre of the organ constitute several large lumina, or the "reservoirs" (Figs. 2 and 3, *rs*), in which the secretion is stored and probably oxydized with light production. The reservoirs communicate also with the outer medium by a number of long, well-defined, parallel tubes, or the "emissory ducts" (Figs. 2 and 3, *dt*). About nine such ducts are generally observed.

The inner walls of the collecting tubules and reservoirs are lined by a low, simple epithelium, while those of the emissory ducts are covered by one which is stratified, a direct continuation of the epidermis of the surface.

Beyond the tubular area—the gland proper—the photogenic organ is enclosed externally by a thick dermal layer in which a great number of chromatophores are found. The latter are especially abundant on the outer surface of the organ. However, they are scattered and do not constitute a pigment cap (Figs. 2 and 3, *chr*).

The blood is supplied by branches of the lower jaw arteries which run near the organ. They enter into the gland proper, passing through the surrounding dermal tissue, and on reaching the tubular area branch out all at once into many capillaries, each making its way between the secretory tubules. After passing the tubular region the capillaries gather themselves again

to form veins which enter into the connective-tissue trabeculæ of the medullary part of the organ.

The tissue of the central part, and to some extent that which encloses the proximal part of the organ, is always loaded with a large quantity of very minute crystals which appear chalky white by reflected light and brown by transmitted light (comp. Figs. 6 and 7, pl. I.).

Harvey states that the luminescence of *Monocentris* is continuous. The fish can produce light both day and night. But the emission of light seems to be controlled to some extent by internal stimuli. The operating mechanism has not, however, been completely ascertained. No special muscle fibers have been found in the organ. Nevertheless it may not be unjustifiable to suppose that the thin stratum of more or less elastic fibers surrounding the outer surface of the tubular region exerts a contraction by which the spontaneous light is produced. Otherwise it is quite difficult to understand the spontaneous luminescence occasionally produced by the fish. This stratum is not clearly differentiated from the ordinary dermal tissue.

I have not been able to detect any nerves entering the gland proper.

Observations on the Living Fish.

In connection with this morphological study on the photogenic organs of *Monocentris* I have made some biological observations on the living fish. The emission of light was seen in darkness under certain conditions. It could easily be produced by agitating the water in which the fish was kept or by adding to it a few drops of ammonia, but very often the luminescence was spontaneous. Luminescence is continuous for even several hours. By rubbing the luminous protuberances with a piece of stick or by scraping them with a knife edge, the luminosity could partially be transferred to the surface of the stick or knife, remaining visible there for several seconds. Although it will be seen that in this case we are dealing with a substance excreted by the organs, it should not be considered that the luminescence is ordinarily due to excreted matter—the "external luminescence"—because no luminous material was seen excreted into the water by a living fish; the luminescence is "extracellular" but "intraglandular."

Monocentris can live in fresh water for a considerable length of time, even for ten hours. Nevertheless, they are quite uneasy under such conditions, as indicated by rapid beats of the fins, stirring movement of the tail, a bubble formation on the skin and, especially, the emission of a continuous bright light.

Monocentris can live in the sea water within a range of temperature between 0° C. and 40° C., and light is produced within somewhat wider limits, the maximum temperature being about 42° C. and the minimum about − 10° C.

Monocentris can show light at any time of the day and night, but the natural luminescence seems to cease in the day time, for the fish after being removed from daylight to darkness did not show this property at least for ten to fifteen seconds.

The regeneration experiment of the photogenic organs, which I have performed, may be interesting and is somewhat suggestive in regard to the problem of the animal luminescence in general. The photogenic organs of eight fishes were excised in varying extent. The wound healed very soon and in the course of about two weeks the integument was completely regenerated. The internal changes following these operations were studied by means of serial sections. The result shows that the tubules of the organs, either completely or partially removed, are not regenerated but are replaced by a spongy tissue consisting of exceedingly enlarged blood vessels. If the granules in the organ were bacterial bodies, they might have been expected to increase in amount, because they were subjected to a favorable condition with an abundant supply of blood. But this does not seem to have been the case. There was no increase in the amount of granular matter in the remaining tubules.

Similar Photogenic Organs of Other Fishes.

Finally the only photogenic organs which at all approach those which have been considered are to be found in two Malayan fishes, *Anomalops katoptron* (Bleeker) and *Photoblepharon palpebratus* (Boddaert) which have been made the subject of memoirs by Steche (1907, p. 85 and 1909, p. 349). A glance at his figures in Pls. XIX–XXI will at once show the fundamental similarity of

the "sub-ocular" organs of the *Anomalopidæ* and the "submental" organs of the *Monocentridæ*. However, the reservoirs and emissory ducts which are well developed in *Monocentris* are wanting in both *Anomalopsis* and *Photoblephalon*. In the latter all of the several secretory tubules communicate with the external medium by one very short common duct. But each photogenic organ in question is an aggregation of many acinous glands developed in a limited area sub-ocular in position, and the secretory tubules stand vertically and lie parallel side by side as in the case of *Monocentris*.

The crystalline deposit is also present. The chromatophores are described as forming a pigment cap which encircles the proximal surface of the organ.

In *Anomalopsis* the oral end of the photogenic organ is said to be fastened to a long cartilagous shaft, upon which it is rotated downwards so as to bring the luminous surface against the body wall and thus cut off the light. In *Photoblephalon* the action of shutting off the light is done by a moveable screen. No analogous mechanisms of light regulation have been found in the submental photogenic organ of *Monocentris*.

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THE DEPENDENCE OF SECONDARY SEX-CHARACTERS UPON TESTICULAR HORMONES IN LEBISTES RETICULATUS.

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I. INTRODUCTION.

The problem of sex as a chapter in the mechanics of development has not been investigated until lately. A number of authors whose investigations were performed on different animals have proven that those sex-characters which are generally called secondary sex-characters depend in their development, on hormones produced in the sex glands. After castration these characters will disappear. Proof to this effect has been produced by Steinach, Sand and Lipschütz in mammals; Pezard, Goodale and Zawadawsky in birds; Harms, Meisenhaimer, Witschi, Bresca and others in amphibians.

The dependence of secondary sex-characters in fishes was not at all investigated until quite recently. The reason for this may be found in the fact that those species which are endowed with permanent dimorphism, the exotic inhabitants of the aquaria, are distinguished by a very scant size and want of stability and therefore, are almost inaccessible for operative intervention.

As to seasonal dimorphism we find in the works of Courrier (2) and Von Oordt (5, 6, and 7) on the stickleback (*Gasterosteus pungitius* and *G. aculeatus*) indirect indications of its dependence on the hormones of the testis.

A direct experiment was carried out by St. Kopeć (4) who succeeded in castrating *Phoxinus lævis* males and females and keeping them alive for three weeks after operation. During this time said fish failed to exhibit nuptial colors at the usual time.

The hybridological analysis of the sex-characters in fishes was carried out by Aida (1) on *Haplochihis latipes* and by Winge (8 and 9) on *Lebistes reticulatus*. Both authors came to the conclusion that the color characters of the male depend on the genes located in the sex-chromosome, especially in the Y-chromosome.

The *Lebistes* female, even if its X-chromosome contains genes of color characters, does not phenotypically exhibit the latter.

II. Description of Material and Methods.

In connection with genetic researches on *Lebistis reticulatus* I had the opportunity to observe a great many of those little fishes in the Moscow aquaria. Among the members of this species as well as among members of other species which have come under my observation I very seldom met with divergencies from usual dimorphisms.

My material consists of seven fishes, six of which are males with marked divergence from typical dimorphism. The seventh is a case of hermaphroditismus glandularis. The malformation of dimorphisms of the males were either inborn or developed later in the life of the individuals. Two males, No. 25 and No. 34, belong to the former class.

1. *Male Fish No. 25.*—The individual measured 37 mm. in length. It was entirely deprived of dimorphic colorations. The shape of the body resembled that of a female. Upon dissection, after the fish died, it was discovered that the testis was entirely atrophied.

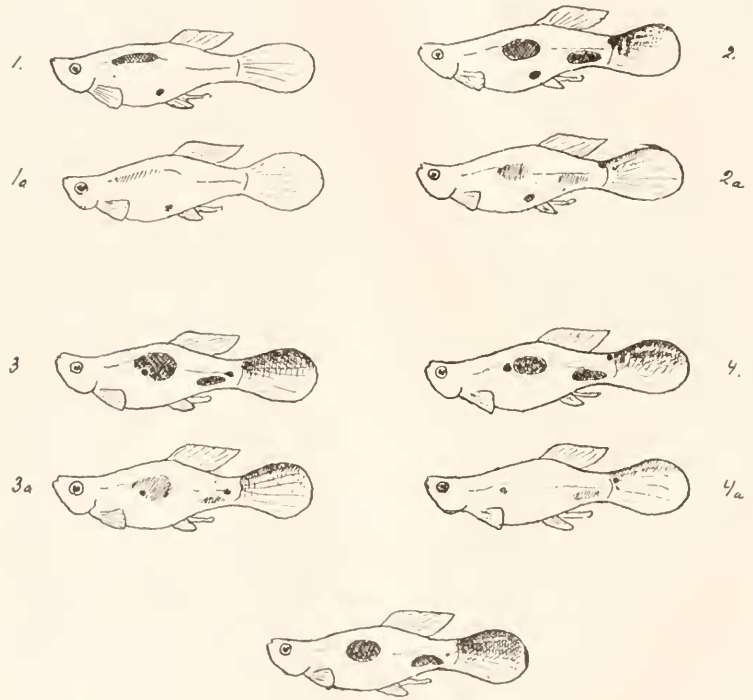
2. *Male Fish No. 34.*—It was born on the 5th of August, 1924. On the 5th of May, 1925, when the animal was nine months old, it measured 23 mm. in length but failed to exhibit any sex-colorations. The gonopod was normally developed. Sex instincts were not lacking; repeated mating to fecundate females, however, proved a failure. No sign of a gonad was discovered macroscopically.

3. *Male Fish No. 23.*—This animal reached maturity in the first part of September, 1924. Up to February of the following year it remained quite normal as to dimorphic colors and sex-instincts. During February the characteristic red spot on the side of the body disappeared and the fish became almost colorless. It died on the 27th of May, 1925, and on dissection revealed entirely atrophied gonads (Figs. 1 and 1*a*).

4. *Male Fish No. 8.*—With normal dimorphism this animal reached maturity in August, 1924. Paleness of sex-colors was noticed in March, 1925. On April of the same year both of the

red spots were almost invisible. Also the black spots found on the side of the body lost their intensity. The specimen died on May 24, 1925. A very slightly developed testis was found after examination of the viscera. (Figs. 2 and 2*a*.)

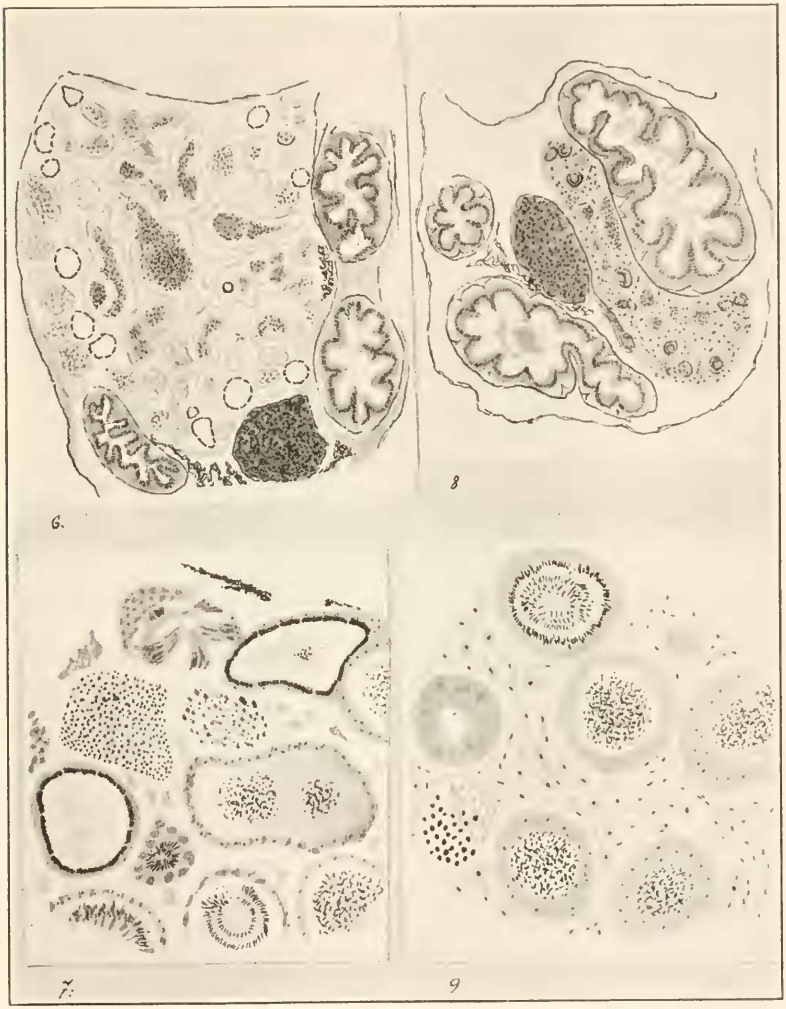
5. *Male Fish No. 33.*—The specimen was born in August, 1924, and reached maturity with secondary sex-characters well formed. From May, 1925, on, the characteristic male shape was under-



FIGS. 1–5.

going a change toward that of the female. This was closely followed by the disappearance of sex colorations. Figs. 3 and 3*a*. On June 4, 1925, it was killed and preserved in formol-alcohol solution. Sections eight microns thick were cut and stained in hematoxylin. Upon examination it was found that the testis was very small and almost deprived of spermatocysts. It contained neither primordial germ cells nor spermatocytes. Spermatogenesis was encountered here and there but only the later stages. The connective tissue of the gonad was well developed.

The coincidence of these two facts—loss of dimorphic colors and the atrophy of the testis—appeared significant and stimulated me to a more detailed study of the last "pale" male No. 44,

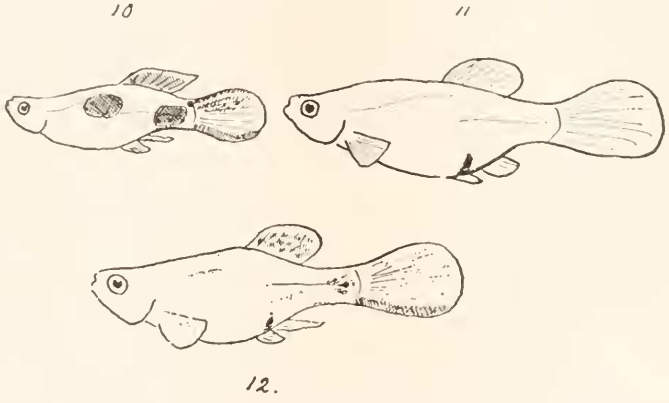


FIGS. 6–9.

Figs. 4 and 4*a*, parallel with its quite normal brother No. 47, Fig. 5.

6. *Male Fish No. 44.*—Phenotypically both fishes, No. 44 and No. 47 were very much alike. Both had two red spots in each

side with the upper part of the tail fin colored yellowish-orange. Male No. 44 had three black spots on each side while No. 47 possessed only one on each side. They were born on the 18th of September, 1924. Both fish developed and remained alike until May, 1925, from which time on male No. 44 gradually lost its coloration and on June of the same year was, so to speak, colorless (Fig. 4*a*). Both males were killed and fixed in formol-alcohol, sections made 6–8 micron in thickness and stained with hematoxylin. The histological picture of the normal male No. 47 showed a large testis and not less than 70 spermatocysts can be counted on one section. The connective tissue is poorly developed (Figs. 6 and 7). In case of male No. 44, the testes is



FIGS. 10–12.

only one fourth as large as that of its normal brother, only 16–17 spermatocysts can be found in one section with spermatogenesis almost reduced to nil. The connective tissue however is very well developed (Figs. 8 and 9). In the middle of the gonad of male No. 44 are found two cysts filled with an almost homogeneous mass. These cysts appear to be much like ova in process of degeneration.

7. *Fish No. 59. The Hemaphrodite*—For this specimen I am very much indebted to Mr. N. J. Dragomiroff, who sent it to me from the University of Kiew. This fish had died 6 months of age and reached me preserved in Tellyesniczky's fluid.

The body of the *Libistes* hermaphrodite measures 37 mm. in length, which is a female characteristic, for the males never exceed

25 mm. The dorsal fin is rounded as is the case in normal females Near the anal orifice a dark black spot can be seen which characterizes the mature female in the viviparous fishes and which has been called "puberty spot" by Essenberg (3). The anal fin however has been modified towards the male direction and resembles a gonopod of not fully developed males, (Fig. 12). The colorations are more like those of the male than those of a female. The black spot found in the tail region is present in the hermaphrodite as well as the orange line on the lower margin of the tail fin. Compare Fig. 10, which is a normal female, Fig. 11,

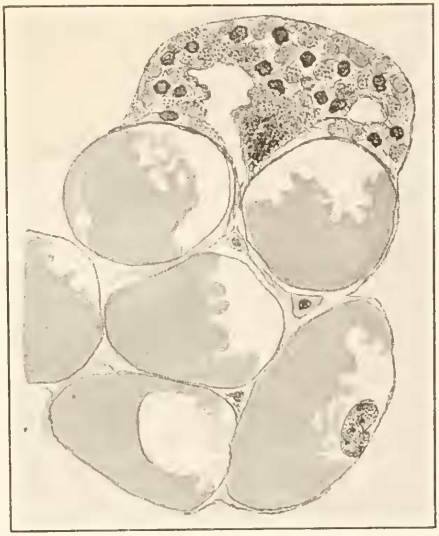


FIG. 13.

a normal male, and Fig. 12, the hermaphrodite. On dissection a fully developed ovary containing almost completely formed embryos was discovered.

The gonad was embedded and sections, 8 microns in thickness, made. Material was stained in Heidenhain's iron-hematoxylin and Mallory's connective tissue stain.

The histological picture corroborates all the features that were observed by the unaided eye, that is, a well-developed ovary with embryos almost ready to be born. Besides all along the ovary, gradually tapering, was found a well developed testicle (Fig. 13).

Contiguous with the testicular tissue lies a mass containing disintegrated ovarian tissue (compare with Essenberg, 1923, Table 5, Fig. 25). In the testicular portion the various stages of spermatogenesis can be found. To all appearance normal and mature sperm cells gathered in cysts (Spermatophores) are just as abundant and regular as can be observed in normal testis.

Discussion and Summary.

It was observed in the description of the six *Lebistes* males that the atrophy of the testis is paralleled by the disappearance of the male sex colors. Based upon these facts the writer concludes that the intensiveness, shape and development of the black and especially the red and yellow pigment spots depend upon the hormones produced in the testis in Lebistes.

With the disappearance of the male sex coloration the individual approaches the colorations of the female, that is to say, the female coloration is the characteristic coloration of the asexual forms.

In the case of mammals, birds and amphibians it is known that the coloration of the asexual forms approaches the sex colorations of that sex which is homozygos as to sex chromosome composition. In *Lebistes*, therefore, the male is heterozygos (XY) as to sex chromosome composition and the female is homozygos (XX).

Winge (8 and 9) has found in *Lebistes reticulatus* the presence of the gene complex S which is located in the X-chromosome and which is transmitted sex-linked. In other species of *Lebistes* (comprising the majority of the species of this genus), the X-chromosome is empty, that is devoid of color factors, and all color characters are transmitted by the Y-chromosome (one sided). The phenotypical manifestation of the S-complex in the males described above consists in the yellowish tinge of the dorsal fin and the orange line along the lower border of the tail fin and possibly the contour of the red spot on the tail.

No doubt the genes of the color pattern of the hermaphrodite belong to the S-complex. These are the orange line along the lower edge of the tail fin, and the red pigment on the tail Any other color characters the genes of which are located in the Y-chromosome, could not be found. Winge states that the Y-

chromosome never is devoid of color factors. If that be so, our hermaphrodite lacks the Y-chromosome and from a genetic point of view it is a female.

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THE RÔLE OF THE NUCLEUS IN THE CELL FUNCTIONS OF AMŒBÆ.

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The importance of the cell nucleus in chemical synthesis of cell elements, regeneration, cell division, fertilization, and heredity is now appreciated. There are, however, many problems connected with rôle of the nucleus in the ordinary, everyday life of the cell which have not been so fully worked out. To many of these problems the metazoan cell forbids an approach because it is too differentiated, too specialized. In addition, it is difficult to eliminate the factor of interrelationship of cells in making such studies on the rôle of the nucleus. For this and other reasons the protozoa have supplied favorite objects for such studies. Because they lead a more or less autonomous existence they exhibit their vitality in a greater diversity of activities than do metazoan cells.

The researches of Gruber, Balbiani, Verworn, Morgan, Hofer, Stolé and many others have supplied ample evidence that an enucleated protozoan is incapable of regenerating any lost cell structure; *e.g.*, Morgan found that as much as 1/64 of a protozoan, *Stentor*, will produce a new individual if it contains a nucleus, but not if one is absent. The non-nucleated part will heal the wound, but will not regenerate lost cell organelles. Verworn too found that if he bisected a Foraminiferan, *Polystomella*, the part without the nucleus would not regenerate the material for a new shell as the nucleated portion did. An apparent exception is the contractile vacuole of amœbæ which, it is generally agreed, always appears anew in an enucleated fragment of an amœba not possessing one at the time of merotomy.

In ciliates generally locomotion is not affected by removal of the nucleus. There are, however, certain factors bearing upon the locomotion, irritability and nutrition of the cell, especially in the amœbæ, concerning which there is much disagreement. The method generally used in studying the bearing of the nucleus

upon any phase of cell activity is by removing it, and observing any variations from the normal in the behavior of the cell.

A. Gruber (1886) was perhaps the first to cut an amœba into two pieces for the purpose of observing the effect of the removal of the nucleus upon locomotion. He says (free translation), "The part with the nucleus continues to project and withdraw its pseudopods, as before but the part without a nucleus withdraws its pseudopods although there is a weak streaming at first. Two days after the bisection the (enucleated) amœba died." He concluded that the experiment showed that in amœbæ the capacity for movement is affected, which he had found was not true in the case of the Infusoria. Hofer (1890) found in a large number of experiments that the removal of the nucleus exerted a direct influence upon the movement of the protoplasm, and he therefore concluded that the nucleus is a regulatory centrum for the locomotion of the amœba. He also found that enucleated amœbæ ceased to form the secretion wherewith amœbæ attach themselves to the substratum. His descriptions and figures show that at no time did the fragments free from nuclear influence approach normal locomotion. Willis (1916) observed a number of enucleated amœbæ for seventy-two hours. He observed that during this time their movements were jerky, irregular and very much slower than those of nucleated parts. They were able to attach themselves to the substratum only weakly and for short intervals of time. Lynch (1919) found that the enucleated part, seven minutes after amputation, ceased its progressive movements and retracted into a corrugated sphere. After one or two days some of the nucleated fragments commenced to move characteristically, but were not able to attach themselves to the slide. Against all this evidence, we have the work of Stolć (1910) who reaches the rather amazing verdict that enucleated amœbæ show the same characteristic movement as nucleated ones, including the feature of attaching themselves to the substratum. Furthermore, he showed to his own satisfaction that enucleated amœbæ were as irritable as nucleated ones, and exhibited the same stimulated condition.

It is evident that it is impossible to gain from the literature any adequate conception of the bearing of the nucleus upon the

locomotion of amœbæ. It was for this reason that the writer undertook the experiments reported in this paper. The amœba employed throughout was *Amœba dubia* which is characterized by an ovoid nucleus, lack of ectoplasmic ridges, and the possession of many pseudopods, with no axial pseudopod. Thanks are due to Dr. J. A. Dawson for giving assistance in way of amœbæ and technique in starting the cultures. After the cultures were once well started, little difficulty was experienced in carrying them on.

From their figures, it appears that Hofer used the type of amœba with the ovoid nucleus, possibly *A. dubia*, while Stolć used an amœba with a biconcave nucleus, probably *A. proteus* or *A. discoides* (See Schaeffer, 1916). The others employed "*A. proteus*," but from their figures it is impossible to determine which species of the *proteus* types.

The instrument employed in my experiments in removing the nucleus was a fine, hard glass point drawn out in a micro-burner, by means of which the portion of the amœba containing the nucleus was severed from the remaining portion. In all cases the enucleated portion was slightly larger than the nucleated. Each portion of the amœba was then placed in a watch glass in a large drop of fluid from a culture in which amœbæ were actively growing, and kept in a moist chamber for observation. The water was changed twice daily so as to be kept as fresh as possible.

Records were kept of sixty-four enucleation experiments, although many more were performed with similar results. The length of life of the enucleated fragments was somewhat variable, as follows:

Two amœbæ lived only one day; eleven, two days; eighteen, three days; sixteen, four days; eleven, five days; four, six days; one, seven days; and one lived eight days. Thus the greater number lived between three and four days. This may be compared with Hofer's enucleated amœbæ which lived from eight to twelve days (one more than fifteen days) and those of Lynch which lived for from six to eight days. Stolć claims to have been successful in keeping amœbæ artificially enucleated alive for as much as twenty-five days. Strangely enough, however, two enucleated fragments which arose from two different amœbæ that had divided spontaneously lived only ten and four days

respectively (Stolć, 1910). It is possible that some of the variations in length of life are due to the species of amœbæ employed by the various workers.

In order to ascertain the effect of the removal of the nucleus upon the locomotion of the amœbæ, sixty-four experiments were performed, and carefully recorded. Each amœba was under observation for a few hours after cutting, and was studied at frequent intervals afterwards so long as it showed any activity. The following experiment is typical of the greater number of the experiments and will be given somewhat in detail.

An amœba moving actively in a healthy culture (Fig. 1) in a Syracuse watch glass was bisected at 3:44 so as to stimulate it as little as possible. The protoplasmic streaming was not discontinued in either piece (Figs. 2 and 3). The fragments moved away, however, in opposite directions from the plane of cleavage, as invariably happens when a clean cut is made so as not to agitate the amœba too much. At 3:45 both portions were still moving, attached to the substratum, as determined by directing a fine stream of water from a pipette upon them. At 3:49 both were still progressing normally. But from 3:49 to 3:54 the activity of the enucleated part slowly diminished (Fig. 4), it lost its hold on the substratum, and the extended pseudopods appeared literally to shrink into short, stubby, wart-like processes (Fig. 5). Under the higher power of the microscope it was apparent that the surface of the fragment was wrinkled and that the clear zone of ectoplasm around the periphery had disappeared, the granular protoplasm extending throughout. Meanwhile the nucleated portion continued to move quite naturally. Later it was placed in a moist chamber where it ingested food and divided two days later. From 3:54 to 5:23 the enucleated fragment remained in the irregularly spherical shape, with the surface considerably wrinkled (Fig. 6). The shape changed somewhat from time to time, but almost imperceptibly. At 5:23 it sent out a long pseudopod (Fig. 7) and commenced a slow streaming, but because it was not attached to the substratum there was no progressive movement. At six o'clock it was attached and streaming actively in an irregular limax fashion (Fig. 8). During the next two days it was attached and streaming actively in a

limax fashion when observed (Fig. 9). On the third day the streaming was slower, change of shape was not so rapid, the attachment to the substratum was loosened, and the periphery of the amœba was a wide zone of clear ectoplasm (Fig. 10). On the fourth day perceptible streaming and change of shape had ceased, and the amœba was called dead.

From the series of experiments, certain definite facts were learned. First, locomotion is not at once affected by the removal of the nucleus if the amœba is not agitated in the process of cutting. Second, from five to ten minutes after enucleation the amœba commences to stream more slowly. This is accompanied by a gradual release of its hold on the substratum. Third, the surface of the enucleated portion becomes wrinkled, as if the pressure from within were reduced. Lynch too mentions the fact that his amœbæ assumed the shape of a corrugated sphere about seven minutes after the removal of the nucleus. Hofer's figures show that he too obtained it. It appears as if the removal of the nucleus causes the cell to lose turgidity. Entire amœbæ placed in 20 per cent. sugar solution become wrinkled in a similar manner after about fifteen minutes. Third, in about two and one half to three hours after enucleation the streaming is resumed, the amœba attaches itself to the substratum, and moves in a manner approximating normal streaming. More often, however, it assumes a *limax* shape, and moves after the fashion of a monopodal amœba, sometimes forming lateral pseudopods, but only temporarily. Fig. 16 shows an enucleated amœba moving in an extremely normal fashion two days after merotomy. Concerning the causes why the amœba should again become active after an interval of comparative inactivity, one can only speculate. There must be a certain amount of pressure within an amœba before it can project a pseudopod. Mast says that it is the contraction of the plasmagel and the hypertonicity of the plasmasol which provides the mechanics of locomotion. The pressure within is reduced when the nucleus is removed, but is increased to some extent after an interval of two to three hours. Certainly then the nucleus is not the centrum for controlling the locomotion of the amœba. Rather, due to some unknown physical cause, its removal seems to affect the imbibition of

water into the protoplasm. Later the property of imbibition is reacquired. If the contractile vacuole is in the enucleated fragment at the time of enucleation, it disappears completely when the fragment becomes wrinkled (Figs. 5 and 6) and does not again appear until streaming is resumed (Fig. 7). Fourth, enucleated fragments, often show a tendency to move in a limax or monopodal fashion. Fifth, the removal of the nucleus does not affect noticeably the ability of the amœba to adhere to the substratum and thus approximate normal locomotion. Sixth, the solation-gelation (Mast 1923) process continues in enucleated amœbæ.

It should be noted, however, that in many cases where to casual observation it appears that an enucleated fragment is moving normally, a closer scrutiny and comparison with a normal amœba shows that the movement is not quite natural. It is difficult to get any quantitative data regarding the degree of normalcy of amœboid organisms. The tendency to move in a *limax* fashion, which *A. dubia* rarely does normally, is the most perceptible deviation from the normal.

There are other irregularities which one finds, such as the tendency of the enucleated *limax* form to flow in one direction for a while, and then very suddenly contract at the anterior end and flow in the opposite direction. Occasionally an amœba will flow in opposite directions from the middle for a short time, in which case the neck of protoplasm connecting the two streaming units becomes very thin. These phenomena seldom occur in normal nucleated specimens.

Stolć has made the assertion, and laid much stress upon it, that enucleated amœbæ show the same irritability as nucleated ones. That is to say, they show what he has designated as the equilibrium condition when streaming unmolested (Fig. 1.), and the stimulated condition when stimulated mechanically (Fig. 11). In experiments like the one described above, the amœbæ were not disturbed for a while after cutting. A number of experiments were performed in which the amœbæ were disturbed just after cutting and at other times. The following is a typical experiment.

At 3:12 an amœba was cut into two approximately equal

pieces. They were drawn up into a pipette and transferred to another watch glass. Both went into the typical stellate stimulated condition (Figs. 11 and 12). At 3:20 the nucleated portion (*A*) was still suspended in the water in the typical stimulated condition. The enucleated portion (*B*) had lost all but two of its processes, and the entire surface of the amœba was rough and wrinkled (Fig. 13). At 3:26 (*A*) was still stimulated, but (*B*) had withdrawn into a wrinkled sphere (Fig. 14). At 3:30 (*A*) was about the same, but (*B*) had sent out a pseudopod with a rough surface. At 3:40 (*A*) was attached and flowing normally. (*B*) was still in a wrinkled condition but with a long wrinkled pseudopod (Fig. 15). At 3:50 both were again drawn into a pipette and shot out again in the stream of water. Both went into the stimulated (stellate) condition, but in just an instant (*B*) became again a roughened sphere. (*A*) remained in stimulated condition for the next ten minutes. At 4:45 both were again drawn into the pipette and shot out in a stream of water. (*A*) showed the typical stimulated condition, but (*B*) went at once into a wrinkled sphere. At no time during the three days of life of the enucleated portion (*B*) could it be made to respond to the stimulus in the normal manner, as did (*A*), which responded in the same manner as the normal entire amœba.

Thus it is apparent that at first both portions show the typical stimulated condition, but the enucleated portion loses the normal response withing a short time. It can respond only by forming a wrinkled sphere, as if it were unable to exert sufficient pressure from within to support the pseudopods. If one watches the "arms" of the stellate enucleated fragment after the initial stimulation following soon after the operation of cutting the amœba into two pieces, the arms appear to melt away, the surface being thrown into folds. The writer can offer no better explanation than that the degree of turgidity becomes insufficient to sustain the pseudopods. Thus we see that the reactions of amœbæ to mechanical stimuli are very much affected, the verdict of Stolé to the contrary. The enucleated fragment has not lost its irritability, but it no longer gives the normal response.

The nutritional processes of amœbæ, especially the ingestion and digestion of food have been further points of controversy.

According to Hofer, Lynch and others, enucleated fragments of amœbæ do not ingest food. Stolć declares that amœbæ ingest food in a perfectly normal manner for many days after enucleation. Schaffer (1920) has pointed out that feeding calls for concerted streaming, and whether enucleated amœbæ ingest food or not is certainly of great importance in evaluating the importance of the nucleus in cell regulation of amœbæ.

Figure 17 shows an *A. dubia* in the normal act of feeding with the interesting observation of one smaller food cup superimposed upon one arm of a large food cup. In culture fluids swarming with *Chilomonas* and *Euglenæ*, the writer has never observed an enucleated amœba ingest a food substance by means of a food cup. It has been observed that such a fragment may flow over a *Chilomonas*, when it appears as if a small cup were formed in the under side of the pseudopod, but on no occasion was the act of enclosing the flagellate and engulfing it into the protoplasm completed.

Schaeffer (1922) has mentioned that by stimulating an amœba with a fine glass rod by waving it in the water some distance from the amœba, the amœba may form a food cup (Fig. 27). While this reaction is not difficult to produce in the case of an entire, healthy amœba, in the case of an enucleated amœba there is either no reaction to a vibrating needle, or only an abortive pseudopod is extended (Fig. 28).

Although enucleated amœbæ which had ingested very large *Euglenæ* could often be found, one was never observed in the act of feeding. Once the writer put a large enucleated fragment of an amœba in a drop of water containing many *Euglenæ* and sat down at the microscope to watch it until the process of feeding should be observed. It was noted that *Euglenæ* which had no flagella often approached the amœba by the typical "euglenoid movement," but the amœba did not react positively toward it. After about an hour it was observed that an *Euglena* was battering the amœba in its attempts to go forward (Fig. 18). Instead of showing an "avoiding reaction," it would simply recoil, and make a new attempt to go forward in the same direction. Soon it had literally battered an entrance into the amœba (Figs. 18, 19, 20, 21). Finally the channel was deep enough to enclose

the *Euglena*. The protoplasm of the amœba closed over the entrance, the *Euglena* struggled a bit, then doubled up, and a food vacuole was formed around it (Fig. 22). Such ingested *Euglenæ* are often digested, although sometimes they are egested very soon after entering the amœba. The process of digestion can be followed. First the *Euglena* gradually loses its green color, becoming brown in an hour or so; then it is reduced in size, and finally after many hours only some brown undigested remains are left in the food vacuole. The vacuole shows the acid reaction up to the end of the process, as proved by its intense redness to a very weak neutral red solution in the water.

Thus, while normal feeding reactions have never been observed in enucleated amœbæ, nevertheless, food may be ingested by an amœba, the organism ingested entering the amœba by its own efforts, and not by any positive activity on the part of the amœba. This method of feeding certainly does not call for concerted streaming. The inability of the amœba to form a food cup seems to be due to the lack of pressure from within sufficient to sustain the pseudopods engaged in the circumvalation of the food particle. Food once ingested can be digested by the amœba after the fashion of normal amœbæ.

In five instances not included in the sixty-four experiments, the enucleated fragment of the amœba had divided spontaneously in from twelve to forty-eight hours after merotomy. The writer chanced to see one such fragment stretched out as in Fig. 23. The streaming was essentially like that of two *limax* amœbæ jointed at their posterior ends. The neck joining the two became narrower and narrower, until the edges rounded off (Fig. 24), leaving two fragments moving in a *limax* fashion (Figs. 25 and 26).

While this is not, of course, normal cell division, it is interesting to note that a cell without a nucleus may divide. The division appeared to be due to the pull exerted in this region by the two halves tending to move in opposite directions, and an accompanying increase of surface tension at the point of division.

Conclusions.

Enucleated amœbæ of the species *Amœba dubia* show the following properties:

1. Within a few minutes after enucleation streaming ceases, and the amœba becomes a wrinkled sphere.

2. In a few hours the streaming is resumed.

3. This streaming is usually of the *limax* type, though it may approximate the normal.

4. Enucleated amœbæ may attach themselves to the substratum.

5. Enucleated amœbæ are irritable, but do not show the same response to stimuli which nucleated amœbæ show.

6. Food organisms which enters the body of an enucleated amœba are killed and digested in an apparently normal manner.

7. Enucleated amœbæ may become divided by antagonistic streaming currents within.

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DESCRIPTION OF PLATE.

(Free-hand drawings of actual specimens, except where stated otherwise.)

1. *Amœba dubia* in normal locomotion.

2. The fragment containing the nucleus, immediately after merotomy.

3. The fragment without a nucleus, containing the contractile vacuole.

4. Enucleated fragment in which streaming is arrested. The hold on the substratum is lost, and withdrawal of pseudopods and wrinkling of the surface has commenced.

5 and 6. The same fragment in the wrinkled condition. The contractile vacuole is not visible in this condition.

7. Streaming and pseudopod formation resumed, but not yet attached to substratum. Contractile vacuole again appears.

8. Streaming of enucleated fragment with pseudopod formation.

9. Limax streaming with small lateral branching, typical of enucleated fragments.

10. Enucleated fragment, practically dead.

11 and 12. Both fragments in stimulated condition.

13. Transitional stage to 14.

14. Wrinkled sphere appearing soon after typical stimulated condition in enucleated fragments.

15. Projection of pseudopod.

16. An enucleated amœba, moving in an almost normal manner, which has ingested and partially digested a number of Euglenæ.

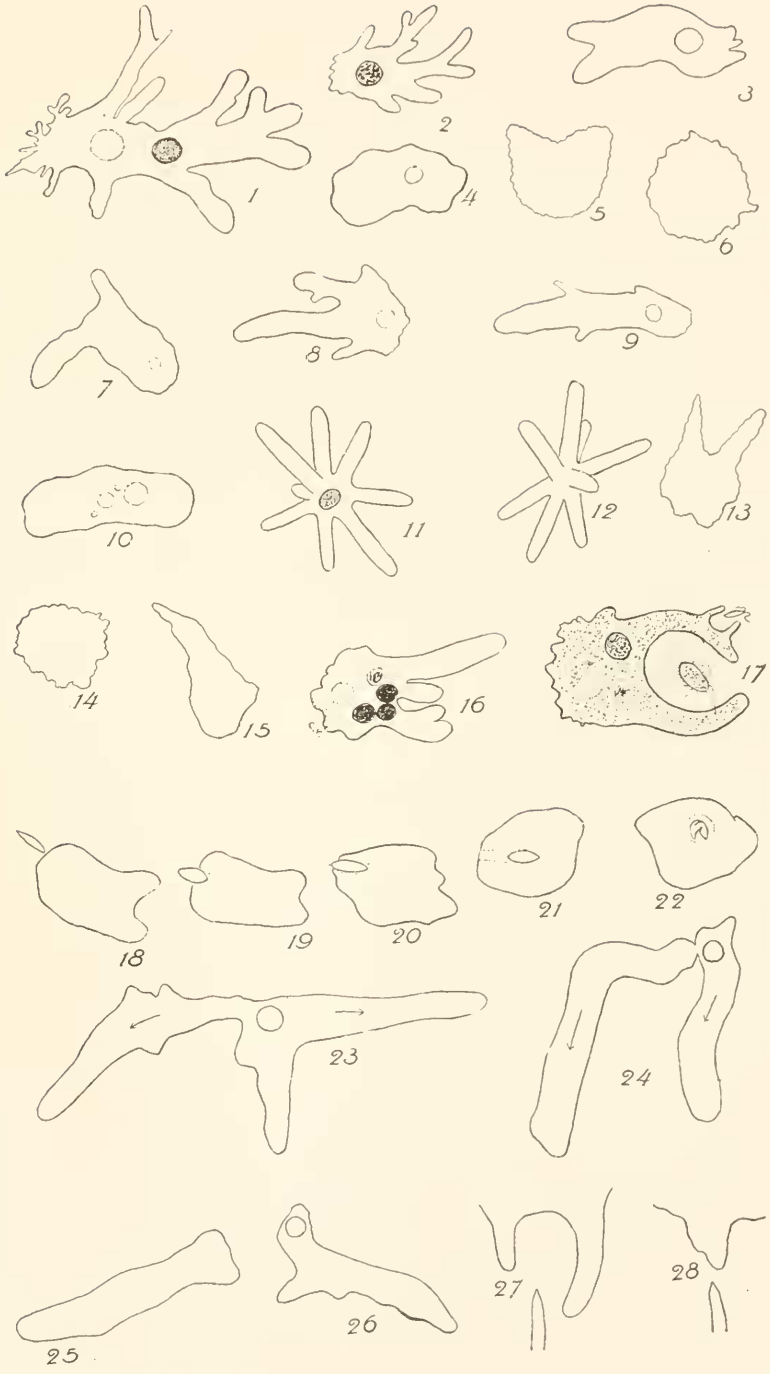
17. Food cup superimposed on a food cup of normal amœba in act of feeding.

18 to 22. An *Euglena* entering an enucleated amœba with final food vacuole formation.

23 to 26. An enucleated amœba dividing autonomously (camera lucida drawings).

27. Food cup induced in an entire amœba by agitating tip of glass rod in water.

28. Abortive reaction of enucleated amœba to the same stimulus.



ELERY R. BECKER.

PALM AND SOLE STUDIES.

IX. The Morphology of the Hypothenar of the Hand; a Study in the Variation and Degeneration of a Typical Pattern.

HARRIS H. WILDER.

Basing our conclusions upon the results of morphological observation, mainly those of Miss Whipple, 1904, it is safe to assume that a friction-skin pattern, whether a "finger-print," *i.e.*, one taken from the apical mound of a finger, or one located upon the broader surface of either palm or sole, has its origin in a raised conical mound, surrounded by an encircling duplicature of skin (Fig. 1). This assemblage of parts becomes covered by series

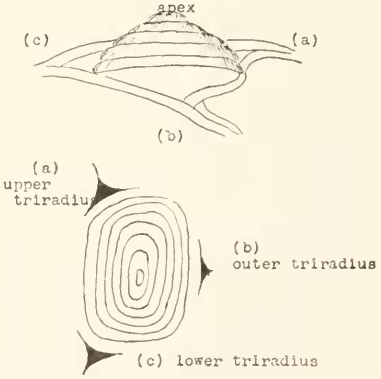


Fig. 1. Diagrams of a pad, and its enclosing folds showing apex, concentric circles and triradii.
Above—Profile view.
Below—Ground plan of same.

of epidermic ridges, themselves composed of rows of single units, probably the morphological equivalents of scales, which in their configuration are influenced by the underlying details. The encircling folds develop as similar ridges which follow the edges, and the two, three, or four points where different folds meet each other form triangular fields, or often triradiate lines diverging from centers, the entire system of which precisely

encloses and defines the pattern areas, upon which the definite patterns are developed. These latter, in typical cases, form in a definite relation to the original conical mound, and develop in concentric circles about the apex of the cone, with the center, or "core," of the pattern exactly coincident with this apex.

When, in the Primates, the height of these structures, that is, folds and mounds, becomes reduced to a flat surface, the relief becomes transformed into a picture, and the mound, with its enwrapping folds, becomes distinctly traceable in the ridges, the pattern area covered with concentric circles, and the outer contour interrupted by triradii. Two radiants of each triradius, extend in nearly opposite directions and embrace, or frame in, a part of the perimeter of the pattern, while a third, the divergent radiant, passes off centrifugally from the pattern.

Those patterns which are located upon the surface of the palms or soles, have each three of these embracing triradii, save the Third of the Interdigital series of the hand, which has four, but the apical patterns, doubtless because of the rounded terminations of the ends of the digits, are furnished with two only, the lateral ones, while the ridges which terminate the digits continue to follow around the contour of the digit, and dispense with a third triradius.

The arrangement of each pattern and its surroundings, including the number of triradii originally embracing each pattern, and their relative position, may be seen by a diagram, published several times, to which the reader is referred.[1] Aside from this the conditions seen in generalized quadrupedal mammals, as shown in the 1904 paper of Miss Whipple,[2] and from which this key diagram was deduced, is of fundamental interest. See especially the paws of *Microtus*, pp. 270 and 272, that of *Crocidura* on p. 280, and the generalized diagram of this author on p. 275, in which each radiant of each triradius is named.

To select a good typical pattern, the transformations of which could be conveniently studied, we may propose the *hypothenar of the hand* (Fig. 2) for the following distinct advantages:

1. It is a pattern with three typical triradii, and consequently three divergents. It is thus better than any finger-print.

[1] "Palm and Sole Studies," Biol. Bull., Feb., 1916, Figs. 3–5, p. 142–144.

[2] *Zeitschr. f. Morph. u. Anthrop.*, Bd. VII., 1904.

2. It is a large pattern, not placed very near any other, and hence so far beyond the influence of others that we are seldom at a loss to decide whether a certain feature belongs to it or to an adjacent pattern.

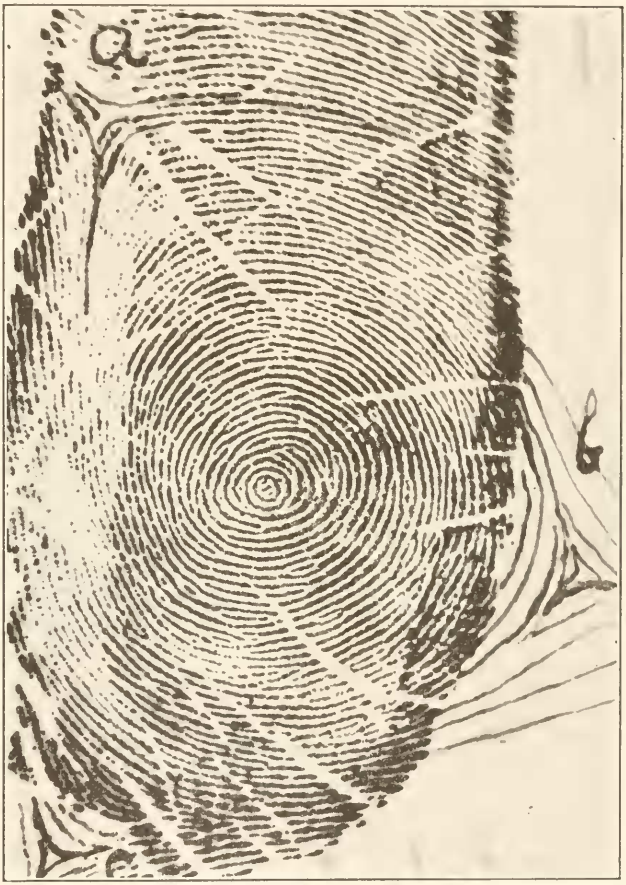


FIG. 2. Actual print of a primitive hypothenar. Triradii *a* and *c* are shown in the print; triradius *b* is indicated by the curve of the friction-ridges and is completed by pencil lines.

3. The region where it occurs is covered by large, heavy ridges, the prints of which are among the most distinct upon the entire palm.

4. This pattern presents an unusual number of variations, and is of fairly common occurrence, rendering it easily possible to collect a large amount of material.

Referring again to any of these diagrams, it will be seen that the pattern in its typical form is centered upon about the middle of the hypothenar region of the hand, and that its three triradii consist typically of (*a*), an *inner upper*, (*c*), an *inner lower*, and (*b*), an *outer*, the first situated near the hollow in the center of the palm, the second down upon the wrist near its middle line, and the third far around upon the outside edge of the hand. This last is, indeed, frequently placed so far around the hand as to be *extralimital*, that is, placed beyond the limits of the true friction-skin, and indicated only by the convergence of the friction ridges along the transition zone between friction-skin and the normal skin of the back of the hand.

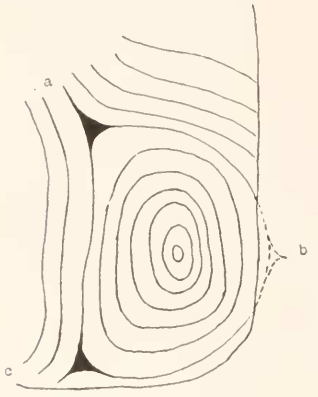


FIG. 3

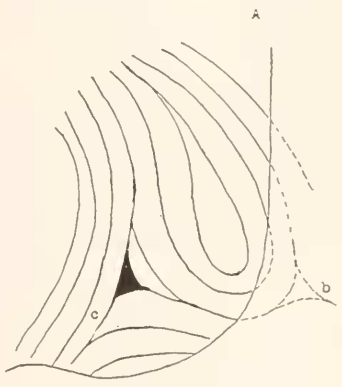


FIG. 4

FIG. 3. A typical hypothenar, based on Fig. 2, an actual print.
FIG. 4. A hypothenar pattern, in which triradius *a* is wanting, and the ridges escape in that direction. This is type *A*.

It is now possible to imagine any one of these three triradii degenerating, and this degeneracy going so far as to allow the escape of the ridges that are normally held in place by its two embracing radiants. Such a loss would convert the concentric circles, a WHORL pattern, into a typical LOOP pattern, open to the point from which the given triradius has disappeared; and as these three triradii are designated as *a*, *b*, and *c*, (Fig. 3) the three resulting loops may receive their names from the triradius that has broken down in each case, and we have types *A*, *B*, and *C*.

In type *A* (Fig. 4) triradius *a* is wanting, while *b* and *c* persist,

thus forming a loop which opens obliquely up across the palm, following the "Line of Life" and pointing up towards the base of the index finger. Type *B* (Fig. 5) opens outwards towards the outer margin, where triradius *b* is wanting, and thus allows the figure to open widely on this side. Triradii *a* and *c* are persistent. In type *C* (Fig. 6) the persistent triradii are *a* and *b*, while the failing one is *c*, the lower medial one. This results in the formation of a loop that runs obliquely downwards and medially, pointing towards the middle of the wrist.

Aside from these three simple types, which result from the loss of a single triradius, there may be found those that result

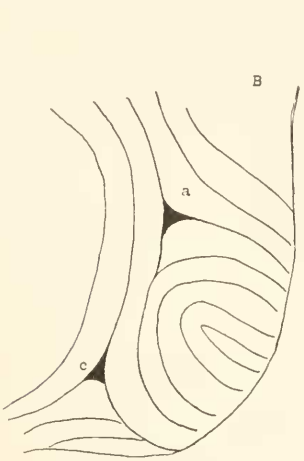


FIG. 5

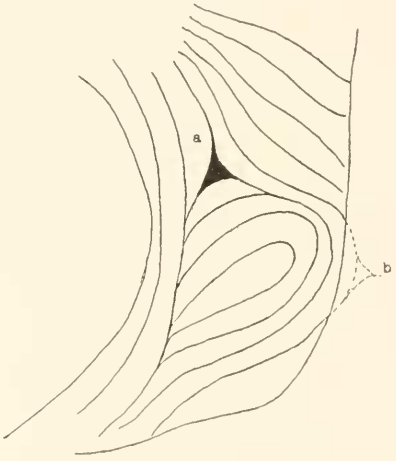


FIG. 6

FIG. 5. A hypothenar pattern, in which triradius *b* is wanting, and the ridges escape in that direction, forming type *B*.

FIG. 6. A hypothenar pattern, in which triradius *c* is wanting, and the ridges escape in that direction, forming type *C*.

from the loss of two out of the three triradii. In these the final result is not a loop, but a lenticular or crescentic figure, tapering at the two ends of a prolonged axis. In type *AC* (Fig. 7) the axis of the figure runs in a curve from above, where it tapers to the hollow of the hand, down to the middle of the wrist, where it tapers again; in type *AB* (Fig. 8) a similar figure is placed obliquely across the hypothenar eminence from upper medial to lower lateral, retaining triradius *c*.

At the present moment I am not quite sure whether type *BC*

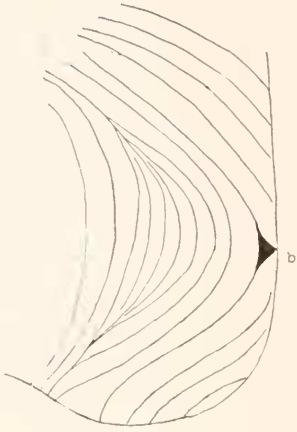


FIG. 7.

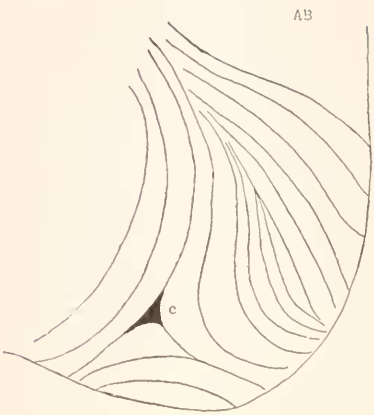


FIG. 8.

FIG. 7. A hypothenar pattern, in which both triradius *a* and triradius *c* are wanting, and the ridges escape in these two directions. This is type *AC*.

FIG. 8. A hypothenar pattern, in which both triradius *a* and triradius *b* are wanting, making type *AB*.

occurs or not, but there seems no reason why it should not. This type (Fig. 9) should retain triradius *a*, the radiants of which

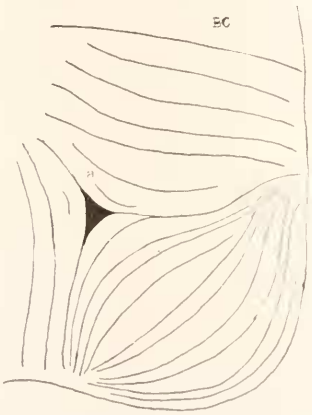


FIG. 9. A hypothenar pattern, in which both triradius *b* and triradius *c* are wanting, forming type *BC*.

should embrace a lenticular figure, shaped like the two others, but with its longitudinal axis running obliquely across the lower outer corner of the palm and tapering down to the points where one normally expects the two triradii, *b* and *c*.

Thus, starting with the primitive whorl, and adding the three

types that arise from the loss of each of the triradii, one at a time, and also the three arising from the simultaneous loss of any two, we have *seven primary types*, in which the variations are due to differences in the originally embracing folds, with their triradii,

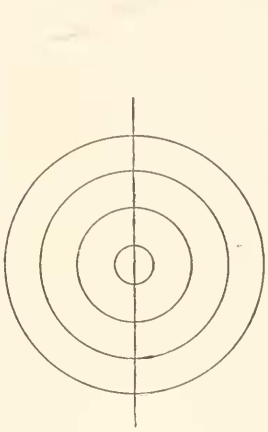


FIG. 10

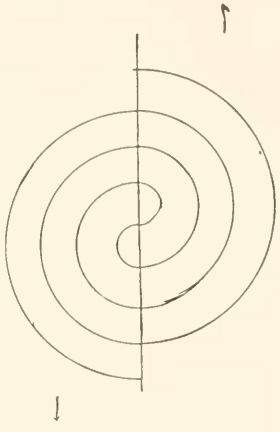


FIG. 11

FIG. 10. A typical whorl pattern, composed of concentric circles. By cutting this through the center, as along the line, and then sliding the two halves as in Fig. 11, an S-shaped spiral is produced.

FIG. 11. An S-shaped spiral the result of sliding the two halves of Fig. 10, as explained in the text. This form is frequent in all kinds of patterns.

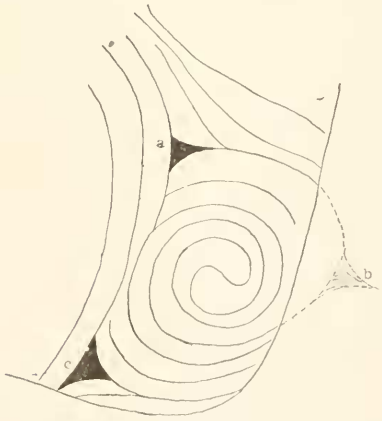


FIG. 12

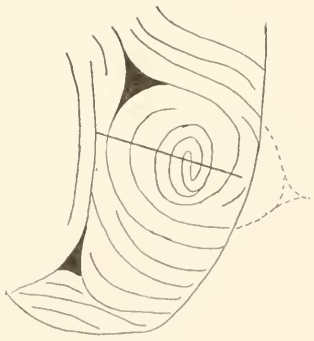


FIG. 13.

FIG. 12. An S-shaped pattern surrounded by its defining triradii.

FIG. 13. An S-shaped pattern, showing the axis along which the slipping may have taken place.

and have nothing to do primarily with the ridges of the pattern itself.

Turning now to the latter there seems to be one distinct line of modification affecting the center of the Whorl; the effect of a shoving of the two halves in opposite directions. This action is most readily seen, and its effect comprehended, by drawing out upon a card a series of concentric circles of the same width, cutting the figure in two through the center, and then slipping the two parts on each other to any distance desired.

Frequently this slipping past of the ridges upon the two sides affects only the central portion of the pattern area, giving a

FIG. 14

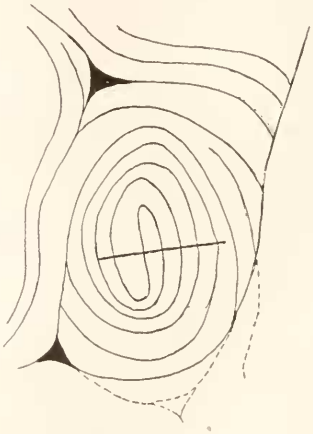


FIG. 15

FIG. 14. An S-shaped pattern, showing an axis of slipping unlike that of Fig. 13.
FIG. 15. An S-shaped pattern, showing an axis of slipping unlike those shown in Figs. 13 and 14.

result quite like an ordinary Whorl, with an S in the center (Fig. 12). In such cases it remains only to be determined the axis of the slipping, that is, the line along which the diagram of Fig. 10 must be cut in order to slip in such a way as to produce the exact pattern we have before us (Figs. 13, 14, and 15).

In other cases, however, where the extension of the slipping spreads the two half-loops further and further apart, the pattern may become spread out so far that the S-shaped figure covers practically the entire hypothenar area, thus producing an actual double figure, or a figure with two distinct loops, and even a

distinct separation between them. In these cases there may appear a new triradius between the two parts, a "degeneration triradius," resulting from this process, and having no counterpart in any element of the primary pattern or its surroundings (Fig. 16).

The two portions of this outspread pattern seem to develop without any correlation; the upper one may remain as a large loop (Fig. 18) or may disappear (Fig. 17, 19). Similarly the

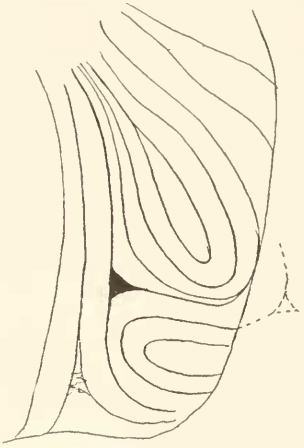


FIG. 16. An S-shaped pattern, which is so large that there has developed a "degeneration-triradius," which cuts the pattern in two and makes two loops of it. Each loop is apparently independent and is directed in different directions.

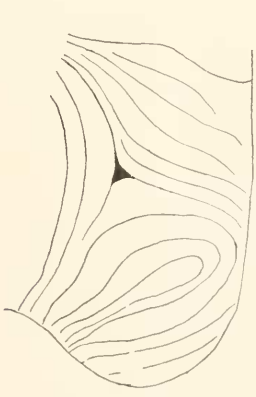


FIG. 17

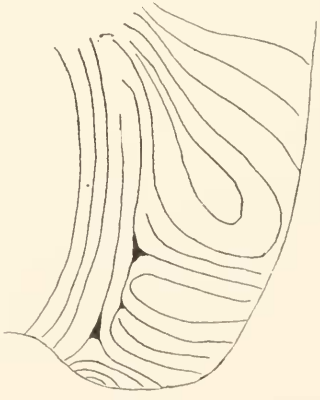


FIG. 18

FIG. 17. A double S-shaped pattern, in which the loops differ in direction from those shown in Fig. 16.

FIG. 18. A double S-shaped pattern with the two loops again unlike those of Figs. 16 and 17.

lower one may appear as a loop, either facing in (Fig. 17) or out (Figs. 18, and 19). Finally the two parts may both become entirely effaced (Fig. 20) the last sign of the former pattern being the degeneration triradius, with the separation of the area of otherwise parallel lines into the two parts of an original S-shaped pattern.

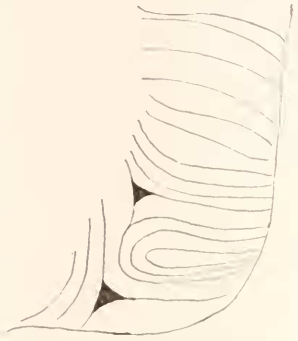


FIG. 19. A double S-shaped pattern, in which the upper loop has disappeared, and is represented by lines that show no trace of a curve.

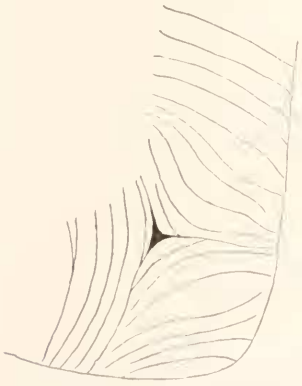


FIG. 20. This shows the ridges covering a hypothenar region, covered by parallel cross-lines, but showing a degeneration triradius, dividing the entire area into halves. It may be supposed that during the evolution of this form it may be evolved from an S-shaped spiral pattern, which has developed a degeneration triradius, and that each pattern of this has degenerated completely.

It is possible to bring into the category of spread-out S-patterns certain cases that consist simply of a rather small and narrow loop, placed very low down on the palm, adjacent to the carpal margin (Fig. 21). This loop suggests the lower half of the double

S pattern, with the loop facing as in Fig. 17, but eventually curving upwards instead of downwards. There may be, in these cases no trace of either a degeneration triradius or of the separation of the whole area into the two halves resulting from an elongated S, and it may be simpler to refer this form to a simple *A* loop of an unusual shape and position.

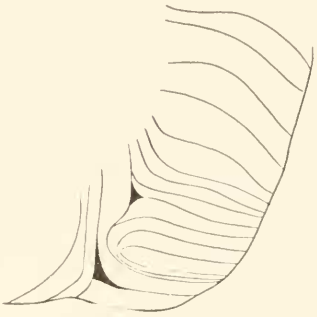


FIG. 21. In this area there is a degeneration triradius, and the lower pattern has retained its loop. This suggests an explanation for a common type, in which the hypothenar region is made of parallel lines, but has a small loop very low down.

The Migration of Triradii.—Thus far we have contented ourselves with the selection, and somewhat with the classification, of types of patterns, and have separated and grouped the following:

W—The Whorl Type (primitive).

A—The *A*-Type, a loop opening upward.

B—The *B*-Type, a loop opening laterally.

C—The *C*-Type, a loop opening downwards and inwards.

AB—The *AB* Type, a lenticular figure, extending between the positions of triradii *a* and *b*, but with the triradii themselves represented only by converging lines, and with the two embracing radiants wanting.

AC—The *AC* Type, same as before but with the missing triradii *a* and *c*, and represented only by converging lines in the two positions.

BC—The *BC* Type, same as the two foregoing, but with the missing triradii *b* and *c*.

S—The *S*-Type, a whorl with an S in the center. This S figure may be confined to merely a few of the central circles,

or may be more extensive. It may be elongated to cover the entire area, and its axis may be placed at any angle.

D—The Two Loop Type, developed from an elongated S, the two parts of which are separate and distinct from each other, and separated by a "degeneration triradius." In subdivisions of this type the two original loops may develop independently of each other.

It has been suggested by the method of description of these types that triradii do degenerate, commonly forming converging lines without lateral radiants; it is also assumed that they migrate in position. In this latter case the movement may be either *centripetal*, the two lateral radiants approaching the center, or *centrifugal*, the lateral radiants allowing more and more complete rings to intervene between the center or core of the pattern and the center of the triradius. In this latter case the two lateral radiants naturally meet at a sharper and sharper angle until eventually they become lost in the set of converging ridges, and are not to be distinguished from the rest. All stages in these two processes are seen in individual cases, although, as no changes are to be found during the life of an individual the process can only be inferred. Thus to start with a case of centripetal movement we can see this only by finding a series of stages, each permanent for a given individual but representing steps in the process carried on by the race. Whether these steps may be found to be taken phylogenetically along a line of descent is not known as yet.

Thus far, in some 3400 human hands, there has been found an hypothenar pattern that does not belong easily to one of the types described here; I found in a Japanese male a hypothenar that cannot well be thus included, and I wish to present it here without further explanation (Fig. 22). It evidently corresponds closely to the types in which the entire pattern is divided by a "degeneration triradius" into two parts; but where in the most of these, each part may be found in the form of either a loop or a completely degenerated area in which it is covered with a series of parallel ridges without trace of a pattern, the upper half of this one, instead of being a loop as might be expected, is in itself a

perfect Whorl, like the pattern we commenced with while below this there is a complete loop. I have looked at this anomalous pattern again and again, without coming any nearer to any explanation. I have therefore given it as it is, an actual pattern which cannot be explained, and the only one out of several

FIG. 22. A wholly excentric hypothenar region found in the left hand of a Japanese. The pattern is double, but instead of each consisting of a loop, degenerate or otherwise, one of them is a loop while the other is a whorl, the type from which we began in the explanation of all double types. It is possible, on the other hand, that we may have here another explanation in which we have to begin with two whorls, divided by a triradius, like the one given here.

thousands. The explanation may occur to some one who reads this paper, or it may well represent a new type, which will require some new modification of the primary pattern; yet it may hardly lead to the abandonment of the fundamental plan of a pattern, which certainly rests upon too much that is fundamental to suggest so radical a procedure.

THE AXIAL GRADIENTS IN HYDROZOA. VIII. RESPIRATORY DIFFERENCES ALONG THE AXIS IN TUBULARIA WITH SOME REMARKS ON REGENERATION RATE.

L. H. HYMAN,

Hull Zoölogical Laboratory, University of Chicago.

Axial Differences in Oxygen Consumption.

The experiments recorded in this section were performed at Swan's Island, Maine, in August, 1924. This island is one of a group off the coast of Maine, opposite Rockford, and is directly south of Mt. Desert Island. My visit to Swan's Island was occasioned through information received from the Anglers Company (now the Denoyer-Geppert Company) that *Tubularia* flourishes in that locality during the summer months, at which season, as is well known, it is in poor condition at Woods Hole. The company further kindly invited me to make use of their collecting station and equipment located on Swan's Island. I am greatly indebted to the company in all of these matters and particularly to Mr. Philip Turner, a member of the firm, for assistance in collecting *Tubularia*.

The collecting station at which these experiments were performed is situated in the town of Swan's Island, which town is located at the head of a large harbor and is provided with the usual wharves, pilings, etc. *Tubularia* was found in large quantities on the pilings of the steamer wharf and is apparently restricted to this one habitat. Members of the Anglers Company informed me that they had never seen it in any other situation nor on any other of the wharves or pilings. Large quantities of the hydroid were readily obtained at low tide from the piles supporting the wharf.

The *Tubularia* occurring at Swan's Island does not seem to be identical with the *T. crocea* of Woods Hole. The stems are much more elongated and branching less frequent. These character-

istics may, however, result from the low temperature of the water, which did not, on the warmest summer days, exceed 15° C. The species is very favorable for experimental work, owing to the long unbranched stems and their freedom from other growths.

For the oxygen consumption large colonies were collected and from these a number of long straight clean unbranched stems were selected. They were carefully examined under the microscope to determine that they were free from diatoms or other organisms. In some cases a few such organisms were seen growing on the perisarc but it was found that these could be removed by gently brushing the stem with a camel's hair brush. The hydranth and upper two or three millimeters and the basal part of the stem were then cut off, leaving a clean stem twenty to thirty millimeters long. This was then cut into two pieces, the basal piece generally taken a little longer than the apical piece to compensate for its smaller diameter. Six to eight such pieces were used in each experiment and an attempt was made to select for each experiment stems of similar diameter. All stems used in any one experiment came from the same colony.

The method of determining the oxygen consumption was the same as previously employed in a similar study on *Grantia* (Hyman, '25). For details this paper should be consulted. Briefly small tubes detachable into two sections are employed. At the end of the experiment the pieces of stem are brought by gravity into one section, which is then removed; the other section is analyzed for oxygen content. In each experiment four tubes are employed: one containing the six to eight apical pieces of stem; one containing the basal pieces of the same stems; and two water blanks.

To compare the rate of oxygen consumption of two objects, it is necessary to know the quantity of protoplasm in each. Owing to the lack of a balance at Swan's Island, the weights of the pieces could not be determined. There would be some difficulty in determining the weight, owing to the presence of the lifeless perisarc around the stems; but some method could probably be devised to eliminate this difficulty. Under the circumstances, however, I was compelled to use the volume of the pieces as a standard of comparison. After each experiment

the diameters of the stems were determined under the compound microscope with an ocular micrometer. However uniform a stem may appear to the eye naturally under the microscope considerable variations in diameter are perceived. Some fifteen to thirty measurements of the diameter of the cœnosarc were made at frequent intervals along each stem and these were averaged. The length of the piece was measured on a millimeter rule. From the average diameter and the length the volume of the piece was calculated assuming it to be a cylinder. No correction was made for the central cavity. The volumes of all of the apical pieces in any one experiment were added together and the volumes of the basal pieces similarly; and when the oxygen consumed is divided by these volumes, the quotients can be used to compare the rates of oxygen consumption of apical and basal pieces.

There is no doubt that considerable error is involved in such determinations of the volume of cœnosarc in the pieces. It does not seem to me, however, that the weight of the cœnosarc could be determined any more accurately. All determinations of the rate of respiratory metabolism are necessarily erroneous since there is no known way of discovering the actual quantity of respiring protoplasm in an organism. The consistent results which I have obtained in *Tubularia*, the definite relation noted between level and oxygen consumption, and diameter and oxygen consumption, indicate that the experiments are sufficiently accurate to render the conclusions acceptable.

Nine experiments were performed. The results are presented in Table I. The first column of figures in this table gives the oxygen content in cubic centimeters of the water in the tube at the beginning of each experiment; the second column the oxygen content in the tube at the end; and in the third column is given the difference between the first and second columns, or the oxygen consumed by the pieces. The data are presented in this way for the sake of simplicity; they are not actually obtained in this form as the original oxygen content of the tubes containing the pieces has to be calculated from the blanks. The differences in oxygen content of the two tubes in each experiment at the start are due simply to differences in the volumes of the

tubes; for the same water is used in both tubes in each experiment. The fourth column of figures gives the total volume of the pieces in cubic millimeters, the fifth column the average diameter of the pieces, and the sixth column the oxygen consumed per cubic millimeter in the time occupied by the experiment. Each experiment lasted four hours. In the first experiment six

Table I.

Showing the Cubic Centimeters of Oxygen Consumed per Cubic Millimeter of Volume in Four Hours by Apical and Basal Pieces of the Stem of *Tubularia*.

Duration of all experiments, four hours.

No. of Exp.	Level of Pieces	O_2 Content at Start, cc.	O_2 Content at End, cc.	O_2 Consumed, cc.	Vol. of Cœnosarc, cu. mm.	Aver. Diam., mm.	O_2 Consumed per cu. mm. in Four Hrs.
4	Apical	.070	.061	.009	32.97	.63	.00027
	Basal	.060	.054	.006	30.84	.59	.00019
5	Apical	.068	.060	.008	24.71	.55	.00032
	Basal	.061	.055	.006	21.90	.53	.00027
3	Apical	.065	.055	.010	20.51	.58	.00048
	Basal	.060	.052	.008	18.68	.46	.00042
6	Apical	.065	.055	.010	20.34	.53	.00049
	Basal	.065	.057	.008	19.61	.51	.00040
7	Apical	.077	.067	.010	18.39	.50	.00054
	Basal	.067	.060	.007	19.25	.48	.00036
9	Apical	.072	.057	.015	20.25	.49	.00074
	Basal	.068	.057	.011	21.76	.51	.00050
8	Apical	.076	.061	.015	17.02	.46	.00088
	Basal	.071	.061	.010	15.08	.44	.00066
1	Apical	.068	.058	.010	11.48	.43	.00087
	Basal	.058	.052	.006	11.10	.41	.00053
2	Apical	.067	.056	.011	13.52	.38	.00081
	Basal	.063	.055	.008	14.59	.35	.00054

pieces of stem were used; in the other experiments eight pieces. The temperature was 20° C. in experiments 1 to 3 and 16° C. in experiments 4 to 9. The volumes of oxygen as presented in the table have been corrected for these temperatures. The nine experiments are arranged in the table in the order of the diameters of the stems used, largest first, smallest last. As already stated,

the six or eight stems in each experiment were selected so as to be of similar diameters.

The following generalizations may be drawn from the data given in Table I.:

1. In all cases the rate of oxygen consumption per unit volume is higher in the apical than in the basal pieces. This result is in harmony with differences in rate of regeneration and in electrical potential which exist along the stem of *Tubularia* (cf. Hyman, '20).

2. There is an inverse relation between the diameter of the stems used and the rate of oxygen consumption, the rate being higher the smaller the diameter of the stems. This agrees with the general law that in animals respiratory rate is inversely proportional to size.

3. In general it appears that the respiratory differences between apical and basal pieces are greater the smaller the diameter of the stem. This indicates that the respiratory gradient is steeper the more slender the stem.

These conclusions are considered further in the discussion.

Experiments on Regeneration.

Data on certain questions regarding regeneration which were obtained on *Tubularia marina* at Pacific Grove, California, in 1922, on *Tubularia crocea* at Woods Hole, Massachusetts, in 1924, and on *Tubularia* sp. at Swan's Island, Maine, in 1924 are herein presented. Owing to the large amount of work that has already been done on the regeneration of *Tubularia*, it does not seem necessary to present these data in detail. For the most part general statements will be made.

The expression "rate of regeneration" is defined and used to mean the time which elapses between the act of cutting and the attainment of a condition of equilibrium. Since in pieces of *Tubularia* of the size employed complete regeneration of a hydranth at the apical end of the piece always occurs, a condition of equilibrium is here synonymous with the completion of a hydranth. Practically, however, it is difficult or impossible to determine the exact time at which the regenerated hydranth is complete. As is well known, the new hydranth forms within the

perisarc and the time of completion of its formation in situ would furnish the most accurate measure of rate of regeneration if such time could be determined. For practical purposes one generally notes the time at which the finished hydranth emerges from the top of the old perisarc. There is no doubt some error in such a procedure for in some cases at least such emergence may be greatly delayed by irregularities in the perisarc through which the hydranth must force its way. However, no better criterion of the time of completion of regeneration has been suggested by anyone and the time occupied by the regeneration process will here be used to signify the time between cutting and the emergence of the completed hydranth.

In all regeneration experiments straight healthy unbranched stems were employed. These were invariably cut as follows. The hydranth and upper millimeter or two and the basal portion of the stem were removed and discarded. The remaining stem was then cut as desired.

The regeneration of only the oral hydranth—*i.e.*, the hydranth which forms at the apical end of each piece—was studied. Unless specifically stated otherwise, all statements refer to this hydranth only. No study was made of the regeneration of aboral hydranths.

1. *Rate of Regeneration of Halves of the Stem.*—In such experiments the stem is divided into halves and the time between cutting and emergence of oral hydranths noted for apical and basal halves. As above stated the observations refer only to regenerated oral hydranths. In a previous paper (Hyman, '20) I presented a considerable mass of data on this matter using *Tubularia crocea*. These experiments showed that the time between cutting and emergence of oral hydranths is markedly shorter in apical than in basal halves. Since writing that paper the same result has been obtained with *T. marina* and with the Maine species. The result on *T. crocea* was also again verified. In Table II, I present a typical experiment on *T. marina*.

2. *Rate of Regeneration of Thirds of the Stem.*—A few experiments on this point were performed on *T. marina* and on the Maine species. The result was found to be different in the two species. In *T. marina*, the apical third regenerates first, the

middle third next, and the basal third last, the differences between the three pieces being well-marked. The basal third in *T. marina* is generally much behind the other pieces. A typical experiment on *T. marina* is given in Table II. On the contrary in the Maine species, the time between cutting and emergence of hydranths is about the same for the middle and basal thirds, the apical third being in advance as usual. I have, however, but five sets of pieces in which the time of emergence of the oral hydranths was exactly determined in all three pieces. These times are as follows, apical third first, middle third next, basal last: 39, 45, 46 hours; 41, 52, 52 hours; 42, 53, 53 hours; 43, 50, 53 hours; and 41, 46, 46 hours. These pieces were 8 to 10 mm. long.

Table II.

Regeneration Rate of Halves and Thirds of the Stem of *Tubularia marina*.

The first column gives the hours elapsed between cutting and emergence of the oral hydranths; and the other columns the number of hydranths emerged at the hours indicated. Length of halves, 3–7 mm.; length of thirds, 5–8 mm.

Hours Since Cutting.	Halves.		Thirds.		
	Apical.	Basal.	Apical.	Middle.	Basal.
29	1	0	1	0	0
30	3	0	1	0	0
31	6	1	1	0	0
32.5	9	2	3	0	0
33.5	12	4	6	0	0
34.5	14	9	8	0	0
35.5	17	11	9	2	0
37	18	14	10	3	0
39	19	16	10	6	0
41.5		18	10	6	0
43		19	10	9	1
44.5			10	9	3
45.5			10	10	4
46.5			11	10	4
48.5				10	6
50.5				11	6
52.5					8
54.5					9
56.5					11

It thus appears that the physiological differences along the stem which are responsible for the differences in regeneration rate at different levels extend further down the stem in *T. marina* than in either of the Atlantic coast species. Banus ('18)

had previously shown a lack of difference in rate of regeneration of middle and basal thirds in *T. crocea*. Differences in the growth habits of the species are in harmony with the experimental findings. In *T. marina* (see illustration in Child, '15, p. 90), there are no branches but the base of each stem runs along the substratum as a stolon for some distance, then turns vertically, and gives rise to a new hydranth at its tip. This method of growth indicates that the hydranth in *T. marina* dominates a considerable length of stem. The Atlantic coast species, on the other hand, branch freely, frequently at a relatively short distance from the terminal hydranth. This fact suggests that the dominance of the terminal hydranth does not extend very far proximally. Beyond this limit the axial differences along the stem would be slight or absent. It is probable that if shorter distal pieces of the stem of *T. crocea* were cut into thirds, a difference would be found in the regeneration rate of the middle and basal thirds. In both my and Banus' experiments, however, rather long stems were used.

3. *Relation of Rate of Regeneration to the Length of the Piece.*— In a previous paper ('20) I reported that the length of the piece has little effect on the rate of regeneration when diameter and level of the distal cut are constant, except when the pieces are very short. This result was again verified on *T. crocea* and on the Maine species. Thus in 16 pairs of pieces of *T. crocea*, the two of each pair being 10 and 5 mm. long, respectively, with the distal cut taken at the same level in both, the 5 mm. pieces regenerated oral hydranths first in eight cases, the 10 mm. first in eight cases. It is understood that the diameter of the apical end was the same in the two members of each pair. At Swan's Island an experiment was performed comparing the rates of regeneration of pieces 15, 10 and 5 mm. long, the distal diameter and level of the distal cut being the same in the three pieces of each set. The rate of regeneration of the 5 and 10 mm. pieces was equal throughout this experiment; and this was also the case with most of the 15 mm. pieces, but a few of them preceded in production of hydranths the shorter pieces by a short time interval.

When the pieces are shorter than 5 mm. in length the production of hydranths is greatly slowed down, so that the regeneration

of pieces 2 mm. in length, for instance, runs far behind that of longer pieces.

4. *Relation of Rate of Regeneration to the Diameter of the Stem.*—It was previously reported that the time between cutting and emergence of oral hydranths is noticeably shorter the more slender the stem. This was again verified on the Maine species. It is reasonable to believe that this result is correlated with the fact, given in the first part of this paper, that the rate of oxygen consumption is higher, the less the diameter of the stem.[1]

5. *Relation of Size of the Regenerated Oral Hydranth to the Length of the Stem.*—A number of measurements were made both at Woods Hole and Swan's Island of the size of the regenerated hydranths on stems of the same distal diameter and cut at the same distal level but of different lengths. Measurements were made with an ocular micrometer, and on the fully expanded hydranth some hours after emergence. The length of the tentacles, the length of the body (distance from tip of manubrium to constriction just below base of hydranth), and width of the widest part of the hydranth (part bearing the proximal tentacles) were measured. The most extensive experiment of this kind was done at Swan's Island. A number of pieces having the same apical level but of different lengths and diameters were cut and placed in one bowl. After regeneration the dimensions of the regenerated oral hydranths and of the original piece were taken. These data are given in Table III. and are arranged with reference to the diameter of the apical end of the piece.

These data show that the dimensions of regenerated oral hydranths are about the same on pieces of quite different lengths but of the same diameter and with their apical ends at the same level. This statement would probably not apply to very short pieces (under 2 mm.) but as shown in the table pieces as short as 2 mm. may regenerate hydranths as large as those from pieces several times as long. Some of the longest pieces may produce

[1] Stems whose diameter is smaller are of course also smaller in other particulars—total length and size of hydranths. As pointed out later they are simply younger as a rule than larger stems. It seems convenient to take the diameter of the stem as a measure of age. Wherever it is stated throughout this paper that the diameter of the piece is smaller it is to be understood that such pieces were taken from stems of general small proportions.

hydranths slightly larger than much shorter pieces but this difference is slight at best and in no wise proportional to the great differences in length of such pieces.

TABLE III.

DIMENSIONS OF REGENERATED ORAL HYDRANTHS WITH REFERENCE TO LENGTH OF PIECE.

Length in millimeters; other figures units of ocular micrometer.

Length of Piece, mm.	Diameter at Apical End of Piece.	Length of Body of Hydranth.	Width of Body of Hydranth.	Length of Tentacles.
2	6.0	15	6	12
5	6.0	18	8	11
9	6.0	21	10	15
2	6.5	14	8	10
5.5	6.5	20	9	12
5	6.5	15	7	12
15	6.5	24	11	17
15	6.5	20	8	14
2.5	6.8	19	8	14
5.5	6.8	22	9	14
5.5	6.8	20	7	10
10.5	6.8	23	12	18
17	6.8	23	12	15
2	7.0	20	7	16
5.5	7.0	18	8	12
5.5	7.0	19	8	13
6	7.0	19	9	14
10	7.0	22	9	14
10	7.0	23	10	15
10	7.0	25	11	15
10.5	7.0	19	9	14
11	7.0	18	8	12
5	7.2	19	8	11
15	7.2	24	10	13
5	7.5	19	10	15
5	7.5	20	9	12
5.5	7.5	20	9	12
11	7.5	20	10	15
11.5	7.5	24	12	22
16	7.5	27	12	19
2.5	8.0	21	8	15
10	8.0	25	12	17
2.5	8.5	22	9	11
5.5	8.5	24	12	12
5.5	8.5	22	9	12
10	8.5	22	11	16

6. *Relation of Size of the Regenerated Oral Hydranth to the Diameter of the Stem.*—Some experiments were performed on this matter. It was found that the size of the hydranth is slightly larger in stout than in slender stems when other factors are eliminated. This difference is detectable only when the difference in diameter of the stems is considerable. In Table III., where there are but small differences in diameter, no definite relation between dimensions of regenerated oral hydranths and diameter of the stems appears. But when stems differing markedly in diameter are compared the dimensions of the regenerated oral hydranths are seen to bear some slight relation to the diameter of the oral end of the pieces. One experiment of this kind is given in Table IV. Stout and slender stems from the same colony, of the same length, and cut at the same apical levels were allowed to regenerate and the resulting oral hydranths measured as in the preceding section. There were ten stems of each lot; these have been averaged in the table for brevity.

TABLE IV.

DIMENSIONS OF REGENERATED ORAL HYDRANTHS WITH REFERENCE TO THE DIAMETER OF THE APICAL END OF THE ORIGINAL STEMS.

Ten pieces in each lot, pieces 20 mm. long. Figures, units of the micrometer scale.

	Diameter Apical End.	Length Body of Hydranth.	Width Body of Hydranth.	Length Tentacles.
	Lot of Slender Stems.			
Min.	5.5	19	8	15
Max.	7.0	26	11	21
Aver.	6.2	23	10	17
	Lot of Stout Stems.			
Min.	7.5	22	11	18
Max.	11.0	30	15	22
Aver.	8.7	25	12	19

Table IV. shows that in the stouter stems the dimensions of the hydranth are slightly larger on the average than in the slender stems. The chief difference is in the width of the hydranth.

The result is readily understandable when it is recalled that in the regeneration of *Tubularia*, the new hydranth is laid down in the old cœnosarc. The new hydranth will then be necessarily broader the stouter the stem.

It will be perceived that the differences in dimensions of regenerated hydranths on stems of different diameter are in no wise proportional to the diameters. In Table IV., the diameters differ by 40 per cent. on the average, while the differences in dimensions of the regenerated hydranths average 10 to 20 per cent. Further, the more slender stems regenerate in a shorter length of time.

7. *Relation of the Size of the Regenerated Oral Hydranth to the Level of the Stem.*—In determining this matter it is necessary that the diameter of the apical ends of the pieces to be compared be the same, for, as shown above, diameter affects the dimensions of the regenerated hydranth. It is a little difficult to obtain pieces from different levels of the same apical diameter since in *Tubularia* the stem generally tapers towards the base. However, it is occasionally possible to find stretches of stem of approximately the same diameter throughout or even some which increase in diameter proximally. Only such have been used in making the comparison. From such stems apical and basal pieces of equal length were cut and after regeneration the dimensions of the regenerated oral hydranths determined. Some data of this kind, obtained at both Woods Hole and Swan's Island are given in Table V.

It is obvious to the eye and measurements also demonstrate that in pieces of the same diameter and length but taken from different levels, the dimensions of the regenerated oral hydranth are nearly always greater on the apical than on the basal piece. Level is thus the most important factor in determining the dimensions of regenerated oral hydranths. These size differences of oral hydranths also of course appear in pieces cut from the usual type of stem, where the diameter of the basal piece is smaller than that of the apical piece; and are too great to be accounted for merely on the differences in diameter.

Table V.

Dimensions of Regenerated Oral Hydranths on Pieces of the Same Length and Distal Diameters but from Different Levels.

Figures, units of the micrometer scale.

Level of Pieces.	Diameter Distal End.	Length Body of Hydranth.	Width Body of Hydranth.	Length Tentacles.
Apical	10.0	15	7	20
Basal	10.0	12	7	16
Apical	5.0	14	5	15
Basal	5.0	10	2	7
Apical	6.5	20	10	23
Basal	6.5	16	7	18
Apical	5.5	30	9	23
Basal	5.5	17	8	27
Apical	7.1	20	10	25
Basal	7.1	17	9	18
Apical	6.6	27	11	45
Basal	6.8	24	11	45
Apical	6.4	28	10	40
Basal	6.4	23	10	35
Apical	6.3	23	7	27
Basal	6.3	21	7	12

Discussion.

The foregoing facts together with others available from the literature support the conception of the existence of a metabolic gradient along the main axis in *Tubularia* and other lower forms; and of a relation between this gradient and the rate of regeneration.

It is shown in this paper that the rate of oxygen consumption in the stem of *Tubularia* is higher in apical than in basal levels. It thus appears that there exists a gradation in respiratory rate along the stem of *Tubularia*. Similar respiratory gradients along the main axis were previously reported for other lower invertebrates: *Corymorpha*, *Grantia*, *Planaria*, several annelids (Hyman, '22, '23, '25, Hyman and Galigher, '21). It is reasonable to believe that they are of universal occurrence among at least the lower Metazoa. We believe that such differences in

rate of chemical activity (with which are doubtless associated other graded differences) constitute the basis of the phenomenon of polarity.

It appears further that there exist in these organisms permanent electric currents whose direction of flow bears a definite relation to the respiratory gradient (for data on *Tubularia*, see Hyman, '20, for other hydroids, Hyman and Bellamy, '22, Lund, '22).[2] This relation is the following: any part of the organism is electronegative (in the external circuit) to any part having a lower respiratory rate than itself. It is probable that the gradation in rate of chemical activity is the chief cause of the electrical gradient. Some biologists are of the opinion that these electric currents constitute a tool, so to speak, which enables one part of an organism to affect another part.

There is some indication that in the hydroids the gradient is steeper in the more apical levels and gradually flattens out basally. This inference is drawn chiefly from the electrical data, the potential difference being greatest in apical levels (Hyman and Bellamy, '22, pp. 332–33, Lund, '22, p. 490); but in *Corymorpha* the respiratory evidence is to the same effect (Hyman, '22). Regardless of the slope of the gradient in distal levels, it appears certain from electrical and other data that the gradient is slight or absent or even reversed in the proximal levels of hydroid stems (see references just given). It follows that at a certain distance from the apical end, the primary gradation practically disappears and new gradations running in the same or the reverse direction may be initiated. The distance to which the primary gradation extends coincides with the limits of the individual and beyond this point buds, zooids, etc., may arise, if the constitution of the protoplasm permits asexual reproduction; or if asexual reproduction is impossible, the basal or caudal parts of the organism may be more or less independent physiologically or nervously of

[2] In a later paper ('25) Lund reversed his statement in the 1922 paper as to the direction of the current in *Obelia*, without offering any explanation of the contradiction. It may be pointed out that Hyman and Bellamy ('22) tested the P. D. along the main axis of colonies of a species of *Obelia* common at Friday Harbor and identified by Professor Nutting as *Obelia borealis* and of *Obelia geniculata* at Woods Hole; and in both species found distal levels electronegative to proximal in agreement with the statement in Lund's 1922 paper but contrary to his statement in 1925.

anterior levels. The existence of an oral or apical end inhibits the formation of any other oral or apical end within the distance limit over which the control of the former extends (see Child, '15, Chapter IV.).

Differences in rate of regeneration (time between cutting and completion of oral or apical structures) with respect to level constitute further evidence of the existence of a metabolic gradient in hydroids. The more apical the level in the whole within the limits of the primary gradient from which the piece is taken the more rapidly does it produce a new apical end. This generalization has been shown to hold in a large number of lower invertebrates, mostly cœlenterates, *e.g.*,—*Eudendrium* (Goldfarb, '07), *Tubularia* (Driesch, Morgan, Child, etc., for references see Hyman, '20), *Corymorpha* (Torrey, '10), *Pennaria* (Gast and Godlewski, '03), *Obelia* (Billard, '04, Lund, '23), *Cerianthus* (Child, '03), *Planaria* (Child, '11), annelids (Hyman, '16). Further proof of the correctness of this generalization with regard to *Tubularia* is presented in this paper. It is also shown that such axial differences in rate of regeneration are independent of size or mass differences at different levels. It can scarcely be doubted that the metabolic gradient is the direct or indirect cause of the apico-basal gradation in rate of regeneration of oral or apical structures.

Another instance of the dependence of regeneration rate on metabolic rate is the difference in these regards between organisms of different ages (sizes). It is shown in this paper and previously (Hyman, '20) that in *Tubularia* the rate of regeneration is more rapid the smaller the diameter of the piece; and further that the rate of oxygen consumption is higher the smaller the diameter. There is thus a correlation between rate of production of oral hydranths and rate of respiratory metabolism; and it is scarcely to be doubted that the latter is the direct cause of the former. It is probable that in *Tubularia* the dimensions of the stem (diameter, total length, size of hydranth) vary inversely with age and that we are really dealing here with age and not size differences. An inverse relation between respiratory rate and age (size) appears to be universal throughout the animal kingdom. In a previous paper (Hyman, '19) I reviewed this matter and

quoted a considerable body of evidence in support of this generalization. Since then I have obtained additional evidence (unpublished) of the inverse relation between respiratory rate and size in *Corymorpha*, starfish, nudibranchs, and tadpoles; and other data have appeared in the literature (*e.g.*, Smith, '25). The relation here found in *Tubularia* between rate of regeneration and age probably also is of general application. Przibram ('07) gives a discussion of this matter and reaches the generalization that the rate of regeneration is more rapid the younger the animal and declines with age.

Not only is there a relation between rate of regeneration (time between cutting and completion of oral or apical structures) and respiratory rate but the amount of tissue regenerated in a given time appears also to be dependent upon metabolic rate. This applies both to axial differences and age differences. In general the more apical the level from which the piece is taken the larger is the size of the oral or apical end regenerated, and the greater the total mass of regenerated tissue. This result cannot be ascribed to differences in mass of pieces from different levels for it also holds when the pieces are of equal mass. It is shown in this paper that in *Tubularia*, the regenerated oral hydranth is larger on apical than basal pieces, when the length and apical diameters of such pieces are the same. Driesch ('99) and Child ('07) had previously noted that the length of the primordium of the oral hydranth is greater the more apical the piece. A similar relation between level and the size of regenerated apical structures or total amount of tissue regenerated was observed by Billard ('04) in *Obelia*, Child ('03) in *Cerianthus* and *Planaria* ('11) and Morgulis ('07) in annelids.

The amount of tissue regenerated in a given time is also greater the young (smaller) the organism, relative to its size. This is shown to be the case in *Tubularia*, pieces from smaller stems producing relatively larger hydranths in a shorter time than pieces of equal length from large stems. A similar relation between age and rate of formation of new tissue was found by Zeleny ('07), Ellis ('08), and Scott ('09).

A third factor appears to be involved as regards the amount of tissue regenerated in a given time. This factor is the degree

of injury relative to the mass of the regenerating piece or organism. Some years ago under the leadership of Zeleny there was considerable interest in this matter. A number of papers dealing with this subject were published (Zeleny, '03, '05*a*, '05*b*, '07, Ellis, '07). A general agreement was reached by the workers in this field that any one part is replaced at a more rapid rate, the greater the amount of tissue removed at the original operation. It is possible that this result also depends on metabolic rate; for every wound is the locus of an increase in metabolic rate and the greater the number of wounds and the smaller the mass of tissue remaining the greater is the stimulation of respiratory metabolism not only at the wounds but also in the adjacent uninjured parts.

In general, then, it appears that the size of regenerated oral or apical structures and the amount of tissue produced in a given time are causally related to the metabolic rate of the regenerating mass.

When metabolic factors such as level, age, or wound stimulation are not involved, the mass of the piece appears to have little or no effect upon the amount of tissue regenerated in a given time. Thus it is shown in this paper for *Tubularia* that pieces differing considerably in length and hence total mass regenerate oral hydranths of equal size in equal lengths of time. Because of this lack of relation between mass and regeneration it can be stated as a generalization that the smaller the original mass (within certain limits of course) the greater relative to its mass is the amount of tissue produced in a given time. Other data in support of this statement will be found in many of the papers already cited.

It remains to consider a paper by Lund ('23) on regeneration in *Obelia*, in which paper certain conclusions are stated which seem to be at variance with those presented here. In the regeneration of *Obelia* as previously noted by Billard ('04) a process grows out from the cut surface and the hydranth differentiates at the end of this outgrowth. Lund has studied the time occupied by this growth process and the rate of elongation of the outgrowth in a series of pieces cut in apico-basal sequence from the main stem of colonies of *Obelia*. Lund finds as did Billard

('04) that the time between cutting and completion of the polyp is shorter the more apical the piece. But according to Lund the time between the beginning and end of the outgrowth is the same at all levels. By defining the regeneration period as the time period during which the outgrowth is elongating, Lund is able to reach the conclusion that the "rate of regeneration" is the same at all levels. It may merely be pointed out that adopting some particular definition in no wise alters the facts of the matter, which are the same for *Obelia* as for other cœlenterates. Lund is able to state that the rate of regeneration does not differ at different levels only because his definition of the expression "rate of regeneration" differs from that used by other workers. The point raised by Lund that the time interval from the beginning to the end of the elongation process is the same at all levels may be correct but it does not seem to me to be proved by his tables and graphs. However, it is difficult to come to any decision on the matter, as neither the time of beginning of growth of the apical pieces nor of completion of growth of the basal pieces is given. It is very probable, nevertheless, that the chief differences in regeneration rate at different levels lie in the early part of the regeneration period. It is admitted by Lund and is shown by his tables and graphs that the rate of elongation of the outgrowth is faster the more apical the level. Billard ('04) had previously made a similar observation; he noted that the sum of the lengths of the outgrowth at both ends of each piece is greater the more apical the piece. In the face of his own data, Lund still attempts to maintain the conclusion that the rate of elongation is the same at all levels on the assumption that the rate of elongation decreases apico-basally because the mass of the pieces decreases in the same direction. He assumes without any proof whatever that the amount of tissue regenerated is proportional to the mass of the piece. Such an assumption is incorrect. As already pointed out considerable differences in length of pieces have no effect on either the time required for regeneration or the amount of tissue produced in that time; and such slight differences in amount regenerated as are correlated with differences in diameter are counterbalanced by the more rapid rate of regeneration of pieces of smaller diameter. All of the available

evidence indicates that the mass of regenerated tissue is not proportional to the original mass of the piece but to the contrary is relatively greater the smaller the latter. In view of all of the facts it is practically certain that the apico-basal sequence in rate of elongation observed in *Obelia* pieces is the result of differences in level. Even though the time occupied by the elongation process may be the same at different levels, as insisted by Lund, still the length of outgrowth produced in that time is greater the the more apical the piece; and hence the "rate of regeneration," even using this expression as defined by Lund, decreases apico-basally in *Obelia* as in other lower forms.

Summary.

1. The rate of oxygen consumption per unit volume of cœnosarc is greater in apical than in basal halves of distal regions of the stem of *Tubularia*.

2. The rate of oxygen consumption per unit volume of cœnosarc is greater the younger the stem (smaller its diameter).

3. The time between cutting and completion of oral hydranths is shorter the more apical the piece in pieces of equal length from distal levels of the stem of *Tubularia*.

4. The statement in 3 may or may not hold for proximal regions of the stem, depending on the species.

5. The time between cutting and completion of oral hydranths is independent of the length of the piece when the apical end of the pieces is taken at the same level, except in very short pieces.

6. The time between cutting and completion of oral hydranths is shorter the younger the stem (smaller the diameter).

7. The size of the regenerated oral hydranths is almost entirely independent of the length of the piece, when the apical end of the pieces is taken at the same level, except in very short pieces.

8. The size of the regenerated oral hydranth is slightly smaller especially as to width the smaller the diameter of the stem but not proportionally smaller.

9. The size of the regenerated oral hydranth is absolutely larger on apical than on basal pieces of equal mass taken from distal regions of the stem.

10. In general there is a relation between respiratory rate and

regeneration. The higher the respiratory rate the shorter is the time interval between cutting and completion of oral hydranths and the larger is the size relatively or absolutely of the regenerated oral hydranth.

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THE VALUE OF INSTINCT AS A TAXONOMIC CHARACTER IN SPIDERS.

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The study of animal behavior under experimental conditions has brought into sharp opposition the supporters of the idea of the rigidity of instinct with the defenders of its plasticity. The inability of the animal, especially of invertebrates to meet new and unaccustomed conditions in human fashion has furnished the chief argument in favor of the attitude of the modern school of behaviorists. The interpretation of an animal as a machine activated by stimuli to reflexes with an outward appearance of intelligence, yet without intelligent control of its actions has been and still is sufficiently often discussed by both parties not to need further discussion here. Some day I hope to put together for print some observations on the plasticity of instinct in spiders, which I have made gradually during the past years. For the present I want to content myself with another aspect of the problem.

The discussion referred to above naturally centers round the phenomena of *individual* behavior. Ascending from individual to species and widening our observations to different geographical regions and environmental conditions, influenced by our preference for the one or the other school of behaviorism we lay stress either on the individual modifications or the specific similitudes of instinct. The same applies of course to generic and family characteristics. The instincts of spiders are apparently particularly rigid in this respect and easily traceable to family relationship in burrowing habits, snare construction, courtship, mating and care of cocoon. Thus all representatives of the family Argiopidæ build orbwebs, all Lycosidæ carry the cocoon attached to their spinnerets, etc. In fact in the earlier days of arachnology the habits were used for family or larger group dis-

tinction and spiders were divided into Terricolæ, Tubicolæ, Citigradæ, Saltigradæ, Laterigradæ, Retithelæ, and Orbithelæ. It was soon recognized, however, that orbwebs are constructed by some spiders anatomically very different from Argiopidæ and since separated from the latter into a family Uloboridæ among the supposedly more primitive division of Cribellatæ. The same was done for the Dictynidæ among the Cribellatæ, which were originally placed with the Retithelæ. The similarity in web construction was explained by the assumption of parallelism in both groups. Similar though not such pronounced parallelism was found in the case of various Tarantulæ or Theraphosid spiders, the habits of which are comparable with those in true spiders. Parallelism of such kind may be easily explained as adaptive to similar conditions of life. But it would be difficult to conceive the construction of such a complicated and perfect snare as an orbweb as a manifestation of habits acquired independently by every genus of Argiopid spiders. The general features of this habit must have been acquired before the splitting up of the original stock into new species and persist notwithstanding the fact that the family spread all over the world from the tropical rain forests to the cold zones of the North and South, and comprises an enormous number of species as unlike each other in size and appearance as *Nephila* with its inch and a half long body and four-inch spread of legs and *Theridiosoma* scarcely a tenth of an inch in length, *Gasteracantha* with her abdomen much wider than long and adorned in some species with long, curved spines exceeding many times the length of the body and *Tetragnatha* with her soft abdomen often many times longer than wide and legs so drawn out and kept in a peculiar fashion that the spider in its web has the appearance of a little twig. All these modifications of structure must have therefore appeared later and influenced only the subordinate features of the habit, the details of the general plan of structure of the geometric web. It was this among other considerations that inclined me to the view which I still hold that the Argiopidæ are descendants of Uloboridæ.

The manner in which the female takes care of the cocoon with eggs is another habit characteristic of whole families. In fact so

distinctive is this habit in some families that on the strength of it the great French arachnologist Simon placed the genus *Rhoicinus* among the Lycosidæ, although neither the disposition of the eyes, nor other important morphological structures are of the type to warrant the inclusion of the former in the very characteristic family of ground spiders. Says Simon in his "Histoire Naturelle des Araignées": "Les quelques espèces, pour lesquelles j'ai proposé le genre *Rhoicinus*, sont très anormales pour la famille des *Lycosides*, dont elles n'ont pas la disposition oculaire; si l'on ne tenait compte que de ce caractère, on les rapprocherait des *Cybaeinæ*, particulièrement des *Campostichomma*, mais leurs trochanters sont entaillés d'une profonde échancrure apicale, leur griffe impaire ne porte q'une seule petite dent basale *et, de plus, j'ai surpris l'une des espèces portant aux filierès son cocon globuleux, comme l'aurait fait un Lycosa.*" (Italics are mine, A. P.) Without discussing the case in question since our knowledge of *Rhoicinus* is as yet very imperfect and I have no first-hand acquaintance with the genus, I merely wish to point out that of the characters mentioned by Simon the notch in the trochanters is a character occurring in other families besides the Lycosidæ, such as Pisauridæ, Clubionidæ, Argiopidæ, etc., the number of teeth in claws is very variable, changing in other families from genus to genus, while the disposition of the eyes is undoubtedly a very old character and remarkably persistent within families, being at the same time very little affected by age. Thus the deciding character in this case remains the cocoon-carrying habit and the question arises, which character is more subject to modification, a fundamental, old morphological character or a certain habit?

On one of my excursions in Porto Rico I was fortunate enough to make an observation which proves definitely that at least the cocoon-carrying habit may be acquired by spiders the morphological characters of which prevent their inclusion in the family Lycosidæ beyond any possibility of dispute. While turning over a rock in a field near Aguas Buenas I noticed two little spiders on the underside of the rock, carrying their cocoons Lycosa-fashion, attached to the spinnerets. Both proved to be new species to which I have given respectively the names of *Bathy-*

phantes ovigerus and *Lithyphantes oöphorus*. The detailed descriptions of the species will be given later in another place, in my study of Porto Rican spiders. *Bathyphanthes* is a well-known genus of the family Linyphiidæ and has numerous representatives in the United States, Europe and other countries. *Lithyphantes* belongs to the family Theridiidæ and has also a wide distribution. Both are small spiders, *B. ovigerus* measuring 2.87 mm. in length, *L. oöphorus* 1.628 mm. Of the former species I have collected later several females and a male under similar conditions not far from Rio Piedras, of the *Lithyphantes* a second female under a cocoanut shell on the beach at Puerto Nuevo Point. Thus I was enabled to make some observations on both species under laboratory conditions. Both behaved much in the same manner.

The cocoon of *B. ovigerus* is globular and rather large in proportion to the size of the spider, being fully 2 mm. in diameter, with thin, white walls and a few eggs well visible through the silk. The spider makes no web of any kind, but walks dragging the cocoon behind her and if disturbed runs for shelter. Deprived of the cocoon the spider shows signs of uneasiness and on discovering the cocoon grasps it with her cheliceræ, bends her almost globular abdomen until the spinnerets reach the cocoon from below, releases her hold on it with the cheliceræ and starts off dragging now the cocoon behind her. For two days she behaved this way. On the third day I was surprised to find her sitting on the bottom of the jar while the cocoon was hanging close by, suspended by a few threads in a small web made of loose threads and much of the type common in small Linyphiids. The explanation of the change in behavior was furnished the same day when minute spiderlings emerged from the cocoon. Toward the end, then, of her maternal duties the original instinct common to all Linyphiids asserted itself, showing that the species still retains some of the family habits and that the new habit did not develop to the point characteristic for Lycosids, where the spiderlings are carried by the mother on her back until they have moulted and are large enough to shift for themselves.

Lithyphantes oöphorus has an elongated abdomen vividly marked with black and white, the colors forming a pattern of a type more or less common for the genus. The cocoon is globular,

still more out of proportion with the size of the spider, being 1.2 mm. in diameter, with thin, white walls barely covering the dozen eggs. The cocoon was firmly attached to the spinnerets and the spider would not release it when disturbed. On the second day, however, she was found sitting on the bottom of the jar while the cocoon was suspended by a few losely woven threads. On examination, it was found that two of the eggs developed into spiderlings, while the remainder were parasitized by an insect. Since the spiderlings emerged from the eggs there is nothing singular in the fact that the mother abandoned the cocoon.

Here, then, we have proof positive that one of the most stable instincts in spiders may be modified and what is still more interesting may be modified in the same sense in two different families, presenting a clear case of adaptive parallelism. It is true that the two families are closely related. In various instances species placed in the family Linyphiidæ were later transferred to the family Theridiidæ, when the presence of the "comb" on the fourth tarsi was ascertained. In the great majority of species the distinction between the two families is quite pronounced and certainly there is no close relationship between the genera *Bathyphantes* and *Lithyphantes*.

It is more difficult to decide which of the two methods of taking care of the cocoon should be considered the more primitive one. At first thought it would seem as if carrying the cocoon attached to the spinnerets were simpler than hanging it up in a web. The spinnerets and the cheliceræ are the organs employed in the making of the cocoon. The former produce and weave the silk, the latter are used in clipping the sheet and in joining the seam. We know spiders which carry the cocoon in their cheliceræ, as for example, *Spermophora* and *Scytodes*. The families to which these two genera belong have been often considered to be primitive. It seems to me however, that the family Pholcidæ to which *Spermophora* belongs, shows all signs of specialization, especially in the structure of the cephalothorax and legs. At any rate, we know as yet no truly primitive spiders which would carry their cocoon attached to the spinnerets, and the Lycosidæ which generally possess this habit, are not in any

sense primitive. For this reason it cannot be possibly maintained that *B. ovigerus* and *L. oöphorus* have simply dropped the habit characteristic of their respective families and reverted to a more generalized and fundamental habit original with all spiders. The conclusion is inevitable that in the course of evolution, before any morphologically considerable changes have occurred, our two species of spiders have modified their habit.

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BIOLOGICAL BULLETIN

FURTHER STUDIES ON THE LIFE HISTORY OF *CRASPEDACUSTA RYDERI*, A FRESH-WATER HYDROMEDUSAN.

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In 1924 I published an account of a study of *Craspedacusta*. This study included the morphology of the medusa and hydroid and the development of hydroids and medusæ from buds. The life history was followed as completely as possible. For these details, the reader is referred to my former paper (Payne, '24). The story remained incomplete as all the medusæ were females. Since the publication of the paper I have continued to make observations in Boss Lake, but have nothing new to add except that medusæ were not found during the summers of 1924 and 1925. The hydroids were still present in 1924, but no efforts were made to find them in 1925. Since the hydroids were present, at least in 1924, conditions must have been unfavorable for medusæ development. Males of *Craspedacusta* were reported by Hargitt ('08) and Garman ('16 and '24). I have been anxious to get the two sexes together and complete the life history. The method which suggested itself was to find the hydroid of one of these male strains and transplant it to Boss Lake, Elkhart. The medusæ live only a short time out of their native habitat, so to transplant them would be impossible. The hydroid, on the other hand, is very hardy. A change of water does not affect it. The main requirement is a food supply.

Through the kindness of Professor Garman, I learned where the medusæ had been most abundant in Benson Creek, Kentucky. This gave me a good idea where to look for the hydroid. As

[1] Contribution No. 211.

noted in *Science* ('25), I made a trip to Benson Creek July 30 and found a few hydroids on the flat stones in shallow water. There had been much rain during the summer in this region and the creek was muddy and most of the rocks were covered with slime. The hydroids were small and were not producing buds. Three weeks later the water had cleared and some of the slime had been washed from the rocks in swift water. Hydroids were found in abundance and transplanted to Boss Lake. They were still small and were not reproducing. No medusæ were found in the Creek. I am inclined to think that no medusæ buds were formed, due to the many rains, the muddy water and slime, and the small supply of food.

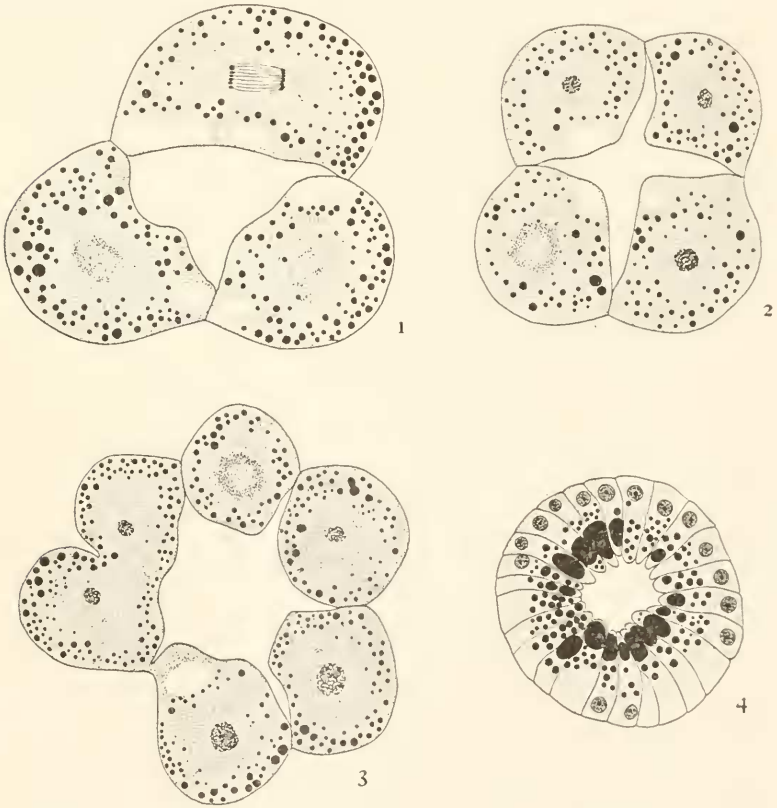
September 11, Dr. W. R. Allen, of the University of Kentucky, wired that medusæ were reported in the Kentucky River. The next day he and I visited the river near College Hill, about 40 miles southeast of Lexington. We found medusæ in abundance at the first place we reached the river, and had no difficulty in collecting several hundred during the course of an hour by dipping them up one at a time. This is not the first time that medusæ have been seen in the Kentucky River. I have learned, with the assistance of Dr. Allen, that Mr. Leonard Giovannoli, now a graduate student in zoölogy at the University of Kentucky, saw them at High Bridge in 1917. This was only one year after Garman's discovery in Benson Creek. High Bridge is 50 miles up the river from the outlet of Benson Creek into the Kentucky River. In 1922 medusæ were seen by Mrs. Alberta W. Server, at Valley View, 35 miles still further upstream. During the past summer, Mr. Wiley Sams, in the course of a canoe trip up the river, saw medusæ at three widely separated places. The uppermost point was near College Hill, which is 40 miles farther upstream than Valley View. Mr. Sams reported the medusæ as very numerous.

As Garman had reported males from Benson Creek and since both sexes had never been found in the same place, I took it for granted that the medusæ we collected were males. Upon arrival in Bloomington with my catch I made an examination of the medusæ and also the eggs which had been shed in the water.

To my surprise I had both sexes and many developing eggs. A week later a second collection from the same place was made by Mr. Giovannoli and brought to Bloomington. They were collected and brought to Bloomington the same day, hence they reached me in good condition. From this lot I was able to work out most of the essential stages in the development of the egg into the hydroid.

Development.

No attempt was made to make a detailed study of the developmental stages, but sufficient work was done to give the main trend of events.

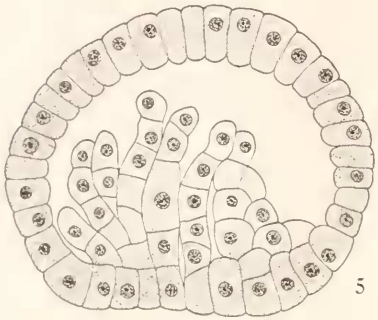


Figs. 1, 2, 3, and 4. Sections of some early cleavage stages; 4, a ciliated blastula.

The germ cells lie in the ectoderm of the gonads, which hang as sack-like pouches from the radial canals. In the ovaries the

eggs form a single layer with small cells wedged in between. In the testes there are many cells showing all stages of development from the spermatogonia to the mature sperm. The first polar spindle is formed before the eggs leave the ovary, but I have never found the division completed. In material from Boss Lake the late anaphase has been seen in a few instances (Payne, '24), but many eggs, in material from Boss Lake and also from Kentucky, show the first polar spindle in metaphase or slightly earlier.

The first cleavage is equal, but after this inequalities enter (Figs. 1, 2, and 3). The rate of cleavage becomes unequal from the two-cell stage. Figure 1 shows a three-cell stage. Even at



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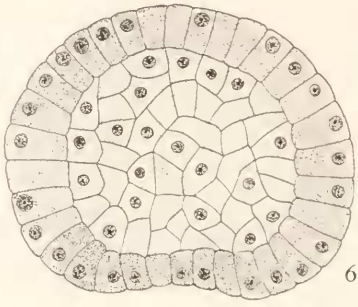

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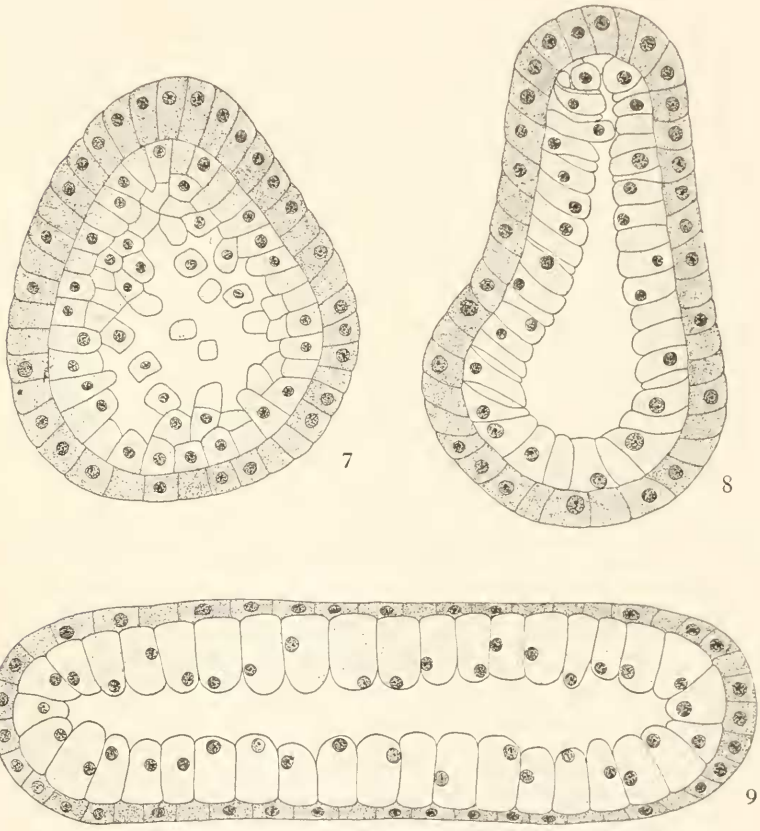
FIGS. 5 AND 6. 5 shows the method of endoderm formation; 6 the solid endoderm.

this time a large cavity is present between the blastomeres. Continued cleavage results in the production of a blastula, the cells of which are long, pointed at the inner ends, and larger at the peripheral ends. The cavity of the blastula is small (Fig. 4). The yolk has accumulated near the inner ends of the cells, so that even in the living blastula the outer ends look clear while the inner ends are opaque. The nucleus lies in the outer clear end. The larva is ciliated and swims about freely at this stage. At what stage the cilia disappear I am not sure, but it occurs before elongation begins.

The method of endoderm formation is similar to, but shows some variations from the process in other Hydromedusæ. There is a slight invagination at one side, probably the vegetal pole.[1]

[1] I shall use the terms "vegetal" and "animal" pole to describe the differences observed rather than to indicate a distinct polarity. I have not oriented the egg to make sure that endoderm formation occurs at the vegetal pole.

At the same time the cells at the place of invagination divide and migrate into the segmentation cavity (Fig. 5). From the figure it may be noted that the cells extend into the cavity in the form of strands. This division and migration continues until the segmentation cavity is filled with cells (Fig. 6). The cells of the outer ectodermal layer are still elongate but less so at the



FIGS. 7, 8, AND 9. 7 shows the beginning of elongation and the formation of the gastrovascular cavity; 8, a continuation of the same processes; 9, the endoderm is now a single layer of cells and the gastrovascular cavity is formed. This stage now attaches at one end. The mouth opening and nematocysts form, thus completing the hydroid development.

All figures drawn to the same scale.

vegetal pole (Fig. 6). This difference between the length of the cells persists throughout the later stages of development. It is

the only method of distinguishing the two ends of the young hydroid (Figs. 7, 8, and 9). Shortly after the formation of the solid endodermal mass of cells, the whole structure begins to change shape. Elongation begins at the animal pole (Fig. 7). This produces a larva somewhat pointed at one end and large at the other. About this same time a cavity begins to form in the endodermal cells (Fig. 7). Presumably this is formed by migration and rearrangement of cells. Elongation continues and along with it the cavity in the endoderm enlarges (Fig. 8). At the end of these processes the larva is practically uniform in diameter from end to end and has a single layer of endodermal cells surrounding a central gastrovascular cavity (Fig. 9). The one distinguishing mark is the unequal size of the ectodermal cells at the two ends. Which of these stages is the typical planula is difficult to say. Dalyell's planula was elongated and ciliated, and the endoderm was still a solid mass. In *Craspedacusta* the cilia are lost and the endoderm is formed before elongation begins. The gastrovascular cavity is formed during elongation. This elongated larva or planula attaches at one end and grows into the typical hydroid. A description of the hydroid need not be given as this was done in my former paper (Payne, '24).

This completes the life history of this interesting form, which for several years has made sporadic appearances here and there, only to disappear without revealing to us much of its story.

Sex.

In all earlier finds of *Craspedacusta*, with the exception of those in Boss Lake, the medusæ were reported to be males. In Boss Lake all medusæ were females. Why were both sexes not found together? In my former paper I answered this question by suggesting that possibly the environment might be a determining factor; also that the hydroids might be male or female producing. It was my inclination to favor the latter interpretation. The fact that we now find both males and females in the same environment would also favor such an interpretation. The sexes are approximately equal in number in the Kentucky River, a count of 110 giving 52 females and 58 males. They are also equal in size in so far as I can judge with

the eye. The only method of distinguishing them is by an examination of the gonads.

Distribution.

In my former paper I stated my belief that *Microhydra* and *Craspedacusta* were merely hydroid and medusa stages of one and the same species, namely *Craspedacusta ryderi*. I also stated that I believed the hydroid much more widely distributed than formerly thought, and that it was only when conditions were favorable that the medusæ appeared. My further studies have strengthened this point of view. No medusæ appeared in Boss Lake in 1924 and 1925. I know the hydroids were present in 1924. No medusæ were present in Benson Creek in 1925. Yet I found the hydroids.

The fact that both males and females occur in the Kentucky River may indicate that this is their point of origin from a marine life. During the elevation of this region, which at times has been a part of the sea, it is possible that *Craspedacusta* became cut off from the main sea and that it was able to adjust itself in the slow change from salt to fresh water.

Size.

The first medusæ taken in Boss Lake, September 1919, were large, measuring from one half to three fourths of an inch in diameter. They were perfect specimens. In later summers they were never so large nor so perfect. I attributed this change to the presence and destructive action of many amœbæ. The medusæ taken in the Kentucky River were large and perfect, similar to the first specimens taken in Boss Lake. I did find amœbæ on them, however, when they were allowed to live in the laboratory until they showed signs of disintegration.

Systematic Position.

The systematic position of *Craspedacusta* has been a debatable one. This is not surprising, however, when we look at the taxonomic work on the Hydromedusæ. A glance at the literature tells one that classification in many cases has been attempted without knowing much or anything of the life history. Hydroids have been classified independently of medusæ and medusæ

independently of hydroids. Originally such studies were necessary, no doubt, but I see no reason why they should continue. Neither do I see how we can arrive at a satisfactory classification until we know the complete life-history of each form studied. This may seem a rather discouraging viewpoint, since we now know the life-history of so few, and when we consider the enormous amount of work and the difficulties involved.

Mayer ('10), in his classification of the medusæ of the world, describes the Leptomedusæ as follows: gonads on the radial canals; otoliths, if present, of ectodermal origin; medusæ arise through alternation of generations from Campanularian hydroids. He describes the Trachymedusæ as medusæ having marginal vela, uncleft bell margins, and lithocyst concretions of endodermal origin. In all textbooks we find statements that Trachymedusæ are without a hydroid stage.

Where then does *Craspedacusta ryderi* belong in our present system of classification? Lankaster ('81), Douglas ('12), Mayer ('10), and others have placed *Craspedacusta* among the Trachymedusæ. Allman ('80) placed it among the Leptomedusæ. On the other hand Günther ('94) described it as a medusa descended from Leptomedusan ancesters, which had developed sense-organs with an endodermal axis independently of the Trachymedusæ. Mayer ('10) regards the Trachymedusæ as transformed actinulæ. He says: "They (Trachymedusæ) commonly develop through an actinula larva in which the bell grows out as a collar-like, or intertentacular lappeted expansion from the sides of the body after the tentacles have appeared, and the tentacles of the actinula become those of the medusæ. The medusa of the Leptomedusæ is formed upon a different plan, for the tentacles grow outward from the bell-margin after the bell has developed. I believe, therefore, that the bell of the Trachymedusæ is not homologous with that of the Leptomedusæ. It is evident that the endodermal otoliths of the Trachymedusæ are not homologous with the ectodermal otoliths of Leptomedusæ. I believe that the medusa-shape has been acquired independently in the *Trachylina* and *Leptolina* forms of veiled medusæ."

Microhydra, along with *Protohydra* and others, has either been placed in a separate subdivision of the Hydromedusæ, or with the Tubularian hydroids.

Since we now know the life-history, we are better able to discuss the systematic position of *Craspedacusta*. First, we find a complete alternation of generations. By this I mean that we have hydroids which give rise to medusæ by means of buds, an asexual process, and medusæ, which, by a sexual process, give rise to the hydroids. If Mayer is correct, it is evident that the medusa of *Craspedacusta* is Leptomedusan in structure and in development, with the exception of the origin of the otoliths. Most certainly it is not a transformed actinula. It arises as a bud from the hydroid. The bell is formed as in the Leptomedusæ and the tentacles are outgrowths from the margin of the bell. The medusa is a typical completely formed medusa when it breaks away from the hydroid. For the details of this process, see my former paper (Payne, '24). I do not see how the hydroid of *Craspedacusta* can be interpreted in any other way than as a hydroid. There are no tentacles, but in other respects it is a typical hydroid and behaves as such. Is it a Tubularian or a Campanularian hydroid? The only essential difference between the two groups is the presence of hydro- and gonothecæ in Campanularia and their absence in Tubularia. The basal half or two thirds of the hydroid of *Craspedacusta* is surrounded by a covering which is in part a secretion, but whether it could be called a hydrotheca and the hydroid classed with the Campanularia is doubtful. If the covering is not a hydrotheca, perhaps the hydroid could be classed as a Tubularian. According to the present classifications there is no such thing as a Trachymedusan hydroid.

The medusa of *Craspedacusta*, with the exception of the endodermal origin of the otoliths, is Leptomedusan. This characteristic is Trachymedusan, but in so far as I can judge, it is the only exclusive Trachymedusan characteristic which the medusa has. Where then, should *Craspedacusta* be placed? I do not see how we can place it in any of the existing groups as these groups are now defined. It would seem to me that it is more closely related to the Leptomedusæ, however. While I do not care to place *Craspedacusta* in either of these groups as they are now, neither do I care to create a new position for it. This must wait for more extensive studies upon the life histories of

the Hydromedusæ. When these are completed we shall be in a better position to make a new classification.

Another structure to be reckoned with in a new classification is the presence of an ocellus-like organ in *Craspedacusta*. It lies at the junction of the outer margin of the bell and velar ectoderm and near the nerve cord. This sense organ (probably tactile in function) is ectodermal in origin (see Payne, '24).

In connection with the taxonomic discussion of *Craspedacusta* it is of interest to note that Joseph ('25) has been able to complete the life history of *Gonionemus*. He followed the development of the egg into the hydroid and also the formation of hydroid and medusa-buds from the hydroid. The hydroid does not transform, as Perkins ('03) was inclined to believe, into the medusa. The life history is essentially the same as that of *Craspedacusta*. Here is a form which has always been placed with the Trachymedusæ. It is quite evident that it does not belong there. Such studies emphasize the need of life-history work before we can hope to make a permanent classification of the Hydromedusæ.

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ENDAMŒBA CITELLI SP. NOV. FROM THE STRIPED GROUND SQUIRREL *CITELLUS TRIDECEM-LINEATUS*, AND THE LIFE-HISTORY OF ITS PARASITE, *SPHÆRITA ENDAMŒBÆ* SP. NOV.

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ENDAMŒBA CITELLI sp. nov.

In the autumn of 1925 the writer made microscopic examinations of the fæcal mass from the intestines of eight ground squirrels belonging to the species *Citellus tridecemlineatus*. These animals were captured alive at various times in the vicinity of Ames, Iowa, brought to the laboratory, and examined at once for parasitic protozoa. In addition to a number of other interesting protozoa, six of the ground squirrels were found to harbor amœbæ in their cœca. In four of the animals the amœbæ were extremely rare, but two showed extremely heavy infections. The movements of the live amœbæ were studied in normal saline solution, and permanent mounts were made by the well-known Schaudinn-iron-hæmatoxylin method.

The amœbæ live within their host in association with myriads of other protozoa. The habitat is limited to the cœcum and the part of the colon immediately adjoining. Localization in the cœcum has been noted by Kessel (1924) in the case of several species of rat and mouse amœbæ. Kessel thinks the PH relationship has something to do with this localization of habitat. Perhaps another factor is the more fluid content of the cœcum which makes a more favorable medium for amœbæ than the colon, where the water content is very much reduced by absorption.

Examination of the substances in the food vacuoles of the amœbæ show that they feed largely upon bacteria and nondescript particles of undigested vegetable matter. The food vacuoles and cytoplasm often contain a curious parasite of the amœba which

resists digestion. This will be discussed below. No red blood corpuscles or tissue cells were ever observed within the cytoplasm. These facts make it fairly safe to conclude that the amœba is a commensal and not a true parasite.

The locomotion of the free forms was studied, because the character of pseudopod formation has come to be of so much importance in correct classification (Dobell, 1921, Kofoid, Swezy, and Kessel, 1923). When kept slightly warmed they exhibited great activity with frequent pseudopod formation. The pseudopods were clear and broadly rounded. The endoplasm did not invade them after they were formed, but they were often withdrawn before the endoplasm had completely filled them. They were not, however, thrust out with the explosive suddenness characteristic of *Endamœba histolytica* which the writer has been fortunate enough to observe on several occasions. Typical "fountain streaming" or limax movement was observed in a number of individuals. The nucleus is not prominently visible in the living specimens, but it can be made out after observing the amœba carefully for a while.

Measurements of stained specimens of the free amœbæ show considerable variation. The smallest one measured eleven by ten micra, and the largest twenty-three by twenty-five micra. An average of ten amœbæ measured was fifteen by sixteen micra, which is somewhat smaller than either *Endamœba coli* or *Endamœba histolytica*, but compares favorably with Kessel's (1924) measurements of *Councilmania decumani* from mice and rats. The nuclei of the free forms measure from four by four micra to six and one tenth by five and two tenths micra in size. Ten measurements averaged four and nine tenths by four and eight tenths micra, which is slightly smaller than the figures given for *E. histolytica* and *E. coli*. (Hegner and Taliaferro, 1924.)

The nucleus is definitely of the vesicular type, with a deeply staining karyosome which varies in position from central (Fig. 6) to extremely excentric (Fig. 1). Usually it is less pronouncedly excentric, as in Fig. 2. This karyosome is surrounded by a clear achromatic zone, which in turn is surrounded by a layer of more or less concentrated slightly basophilic substance (Figs. 1–6; 8). Between this layer and the nuclear membrane is an

achromatic reticulum on which are suspended fine basophilic granules. The achromatic nuclear membrane is encrusted on the inner surface by a fine beading of chromatin granules, which is intermediate in coarseness between *E. histolytica* and *E. coli*. A few binucleate individuals were found, but no division figures.

Cysts were exceedingly rare, and only four eight-nucleate cysts could be found; but these were well stained and suitable for study. The shape is nearly spherical (Fig. 9); size, fifteen and one half micra in diameter. Their especially characteristic feature was the thickness of the cyst wall, which was in all cases about one micron. This compares with a thickness of less than 0.5 μ in *E. histolytica* and *E. coli*. The nuclei likewise were fundamentally different from those of the other *Endamœbæ*. All the chromatin from the karyosome and periphery of the nucleus appeared to have collected into a number of irregular, deep-staining blobs. Some of these lay upon the nuclear membrane, while others were farther in the interior of the nucleus. The nuclei of the cyst measured about 2.8 micra in diameter. In contrast to the free forms, where it is coarsely alveolar, the cytoplasm of the cyst appeared granular. Chromatoid bodies were absent, except for a few small dark staining splinter-like bodies in the center of the cyst. The developmental stages of the cyst were not found.

It was upon the basis of thickness of the wall of the cysts, its yellowish tinge, and the character of the cyst nuclei that a new species was created for this amœba. Were it not for this characteristic cyst, it would be difficult to distinguish this from many other *Endamœbæ; e.g., E. muris* (Grassi, 1882) of the mouse and rat.

The writer in a previous paper (1922) pointed out that simply finding a parasite in a host where it had not previously been found was no valid reason for considering it a new species. Especially is this true in the case of amœbæ; for if, as Kessel (1923) determined in his experiments, rodents can be infected with the human amœbæ, it is probable that man can be infected with the amœbæ of rodents. Two points are so sufficiently clear that they should not be ignored in future work in amœbæ. First, is the species being considered sufficiently different morpho-

logically from closely related species so that it should be considered a new one? Second, there is the possibility of man, or domestic animals, becoming the host of an endamœbæ normally found in lower animals. Kessel's work on specificity has encountered severe censure, especially from European workers (*e.g.*, Wenyon). It seems to the writer that the more logical method to criticize the work would be to repeat it, and thus determine if it is really open to such serious defects as has been charged.

Sphærita endamœbæ sp. nov.

The name *Sphærita* was given by Dangeard (1886) to a genus of the family Chytridiaceæ, which he considers to represent a transition from animal toward plant forms. Likewise, Doflein (1916) assigns these forms to the borderline between the plant and animal kingdoms, with the additional comment that they must be reinvestigated by one who would study the relationships of the sporozoa, flagellates, and rhizopods. They are of interest to protozoölogists not only because of their phylogenetic relationships to the protozoa, but also because *Sphærita*, and other cythridines, such as *Nucleophaga* (Dangeard, 1895), are parasites upon protozoa, *Sphærita* in the cytoplasm, and *Nucleophaga* in the nucleus. A number of the earlier observers, particularly Stein, Carter, Kent, and de Lanessan misinterpreted the developmental phases of a *Sphærita* within *Euglena* as the production of embryos from the nucleus of the *Euglena*, which grew flagella and later developed into the adult flagellate (see Dangeard, 1886). Dangeard (1886, 1894, 1895) clearly showed that what these authors considered to be the growing and multiplying nuclei of *Euglena* were in reality cythridine parasites of the flagellate for which he established the genus *Sphærita*.

There are not many references to *Sphærita* in the literature. Dangeard proposed the name *Sphærita endogena* for the form found in flagellates (*Euglena*, etc.) and rhizopods (*Nuclearia* and *Heterophrys*). Later Chatton and Brodsky (1909) proposed a separation of the species found in these two groups of protozoa, suggesting that *Sphærita endogena* be retained for the form found in rhizopods, and that the *Euglena* parasite be called *Sphærita dangeardi*. Chatton and Brodsky (1909) described a

Sphærita from *Amœba limax* Dug., which they found to be different morphologically in the younger developmental stages from *S. dangeardi*. No comparison was made with *S. endogena*. Dobell (1919) mentions a *Sphærita* in the free forms of the parasitic amœba, *Endolimax nana*. Kessel (1924) found a *Sphærita* in *Councilmania muris*, entozoic in mice and rats. There are a number of other papers on *Nucleophaga*, closely related to *Sphærita*, except that it is found in the nucleus. It was originally described from *Amœba verrucosa* by Dangeard (1895), but we will not discuss this genus any further here.

The greater number of *Endamœba citelli* from one ground squirrel were parasitized by a species of *Sphærita*. This material showed so great an abundance of individuals in different stages of development that it has been possible to follow almost the complete life-cycle of this interesting cytozoic organism. The earlier stages of development of the parasite were the first to be seen within the cytoplasm of the amœba (Figs. 2, 3, 4). The first impression was that they represented nuclei in the process of construction from chromidia. Further search revealed the large plasmodia with maturing spores (Fig. 7), which led to the correct identification of the bodies as stages of the life-cycle of a *Sphærita*. It would not be surprising if intracellular parasites of protozoa have led observers astray more often than is generally known; *e.g.*, Leidy in Plate VII. of his "Fresh-water Rhizopods of North America" figures a number of specimens of *Amœba villosa* with "large and coarsely granular nuclei," which "nuclei" were probably typical sporangia of a *Sphærita*, the "coarse uniform granules" being the spores. This interpretation is strengthened by Fig. 15 of the same plate, which Leidy describes as representing "collapse of the contractile vacuole and the bursting of one of the nuclei with the simultaneous escape of the granules or spores of the nucleus and the contents of the contractile vacuole." What he probably observed was the liberation of the spores from a sporangium of *Sphærita*.

The life-cycle of *Sphærita citelli* can perhaps best be described by referring frequently to the figures of the plate. Fig. 2 represents an amœba with two parasites in the earlier stages of development. The lower one has a fine cell membrane,

which encloses a centrally located, deeply-staining nucleus. The nucleus shows no nuclear membrane or other differentiation, and its diameter is about two thirds that of the cell. The nucleus of the upper specimen has just divided with no apparent spindle or attraction spheres. At this stage the opposing surfaces of the two nuclei are flattened, with the remaining surface of each nucleus convex. The uninucleate and binucleate stages of the cell are about the same size, measuring from 1.9 to 2.5 micra.

A second bipartate division provides the plasmodium with four nuclei with the planes of both divisions still plainly marked (Figs. 3, 4, 18). The organism has become more oval in shape and has increased in size to about 2.5 micra in width and from 2.8 to 3.3 micra in width. From this stage the divisions of the nuclei are not necessarily simultaneous. Specimens were found with eight nuclei (Fig. 5), or with six nuclei, four of them smaller and resulting from the division of two of the nuclei of the four-cell stage, with the other two larger and still undivided (Fig. 19). The plasmodium at this stage measures from 3.7 to 4.0 micra in width to from 4.2 to 5.3 micra in width. Divisions are multiplied until the multi-nucleate spherical stage is attained (Figs. 6, 20, 21). These spheres measure from 5.3 to 8.8 micra in diameter.

The nuclei of the spheres just described stain uniformly black. The next stage in the cycle is the transformation of these nuclei into spores. In this process they enlarge somewhat, stain less intensely, and form a definite spore wall (Figs. 7, 22). Some of them show a thickening of the wall on one side (Fig. 22). These spores usually vary in size from 1.0 to 1.6 micra in diameter. The larger spore in Fig. 22 is exceptionally large, measuring about 1.8 micra. Occasionally spores no larger than 0.5 micron in diameter are found.

A comparison of *Sphærita endamœbæ* with the *Sphærita* from *Amœba limax* so carefully described by Chatton and Brodsky (1909) shows certain fundamental differences. First, the nuclei of the young uninucleate forms are comparatively large and central in *S. endamœbæ*. Those from *Amœba limax* were punctiform and excentric. These distinctions alone are sufficient to justify a distinction between the two species. Second, not all the nuclei of *S. endamœbæ* develop simultaneously into spores,

as they apparently do in the form from *A. limax*. Third, there is no nucleus present within the spores of *S. endamœbæ*, although the contents of the spore are clearly visible. Chatton and Brodsky state that it was difficult to see the interior of the spores of their *Sphærita* even in stained specimens. In a few cases, however, they observed an excentric nucleus within the spore. Fourth, the appearances of dividing nuclei of the multinucleate plasmodium dffer in the two species. Those of *S. endamœbæ* are bilobed, or dumb-bell shaped. Those described by Chatton and Brodsky presented the appearance of two cuneiform polar caps. Measurements of the two species in various stages lie within approximately the same limits. The above stated facts make it evident that the *Sphærita* of the *Endamœbæ* is altogether different from that of the free-living amœba, *A. limax*.

Likewise I believe it is different from the one figured by Dobell (1919) in *Endolimax nana*, an amœba entozoic in man. If his figures be correct, the nucleus of the uninucleate stage is punctiform and excentric, as in the parasite of *A. limax*. The morula-shaped mass of spores is likewise not characteristic of *S. endamœbæ*. Kessel's (1924) account is too meagre to afford a comparison.

Chatton and Jansky were not able to determine whether the spores of their *Sphærita* became flagellated, after the manner of the zoöspores of *Sphærita endogena* and *S. dangeardi* as described in the accounts of Dangeard, or remained immobile and were passively ingested by the amœba. Although actual reinfection by the spores was not observed, the writer has been able to follow out the process in his prepared slides.

Among the bacteria present on the slide one occasionally finds dumb-bell shaped organisms, resembling *Azotobacter*. There seem to be two general sizes, one considerably larger than the other (Fig. 10). These multiply by binary fission (Fig. 11). It is not unusual to find these dumb-bell-shaped bacteria-like bodies in the food vacuoles of the amœbæ (Figs. 8, 12, 13). In the food vacuoles the organism undergoes considerable change. Dense granulation appears in the more or less homogeneous cytoplasm. The dark granules collect in a deeply-staining clump in the center of the cell (Figs. 3, 8, 14). The two members of the dumb-bell shaped pair usually separate, and are carried some distance from

each other. Finally, the deeply-staining mass of granules becomes compact and uniformly solid (Figs. 2, 3, 8, 15, 16, etc.). By this time the fluid content of the vacuole has disappeared and the wall of the *Sphærita* is contiguous with the cytoplasm of the amœba. The development then proceeds as described above.

The writer realizes that it may be objected that two organisms have been confused in this cycle, and that what has been interpreted as the infective form of the *Sphærita* is in reality a bacterium ingested as food. This danger was a cause of considerable anxiety, and it was not until a large amount of material was studied that the writer was convinced of the specific identity of the two forms. A careful study of such appearances as in Fig. 8 (where one finds a perfect series from the bacterium-like form to the early uninucleate form, unmistakably that of *Sphærita*) brings conviction of the transition from one form to the other. The only gap in the life-history is the failure to observe convincing stages of the growth of the spores into these larger bacterioid forms. It is to be expected that such stages would be exceedingly difficult to find, considering the amount of the fæcal mass in proportion to the number of spores. The writer has observed, however, the smaller dumb-bell-shaped dividing spores resembling the smaller individual in Fig. 10 within an old sporangium from which all but a few of the spores had been expelled.

The extracellular development of *S. endamœbæ* differs in several important respects from that described by Dangeard for the forms which he found in free-living flagellates and rhizopods. Here the zoöspores became elongated and flagellated as they left the sporangium. Then they united in pairs, as in conjugation. The spores studied by the writer were never flagellated when found outside the amœba, and no conjugation of spores was observed, although what appeared to be dividing spores indicating a free multiplication cycle were often found.

As in the case of *Sphærita* living in other protozoa, this *Sphærita* is mildly pathogenic to its host. Most parasitized amœbæ exhibit no degenerative changes of any kind (Figs. 2–6, 8). Some of the more heavily infected ones, however, manifest the ill-effects of parasitism by abnormal nuclear appearances (Fig. 7). The karyosome becomes swollen and irregular in shape.

The chromatin beading on the nuclear membrane collects into thick, elongated, deeply-staining blobs. Dobell (1919) also figures nuclear degeneration in parasitized *Endolimax nana*.

Summary.

1. *Endamœba citelli* sp. nov. is a commensal in the cœcum of the striped ground squirrel, *Citellus tridecemlineatus*.

2. The nucleus of the free form is typical of the genus *Endamœba*.

3. The cyst is characterized by a nuclear structure somewhat different from that known for other amœbæ, and an unusually thick cyst wall with a yellowish refraction.

4. The cytoplasm of this amœba may contain a cytridine parasite, *Sphærita endamœbæ* sp. nov.

5. The developmental cycle of this cytozoic parasite was followed from the free bacterium-like infective stage to the spore liberated from the sporangium inside the cytoplasm of the amœba.

6. *Sphærita endamœbæ*, like other members of the genus, produces degenerative changes in the protoplasm of its host, particularly in the nucleus.

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EXPLANATION OF PLATE.

× 1420.

FIG. 1. Free form of *Endamœba citelli* with typical nucleus fixed with two pseudopods extended. Two food vacuoles in cytoplasm contain respectively a bacterium and a partially digested yeast.

FIG. 2. Amœba infected with young uninucleate and binucleate *Sphærita*.

FIG. 3. Amœba with same stages plus one younger individual with nucleus in process of formation and an individual with four nuclei.

FIG. 4. Large amœba with one parasite having four nuclei.

FIG. 5. *Sphærita* has eight nuclei, each dumb-bell-shaped preparatory to division.

FIG. 6. Multinucleate *Sphærita* in cytoplasm of amœba.

FIG. 7. Large sporangium with nuclei developing into spores.

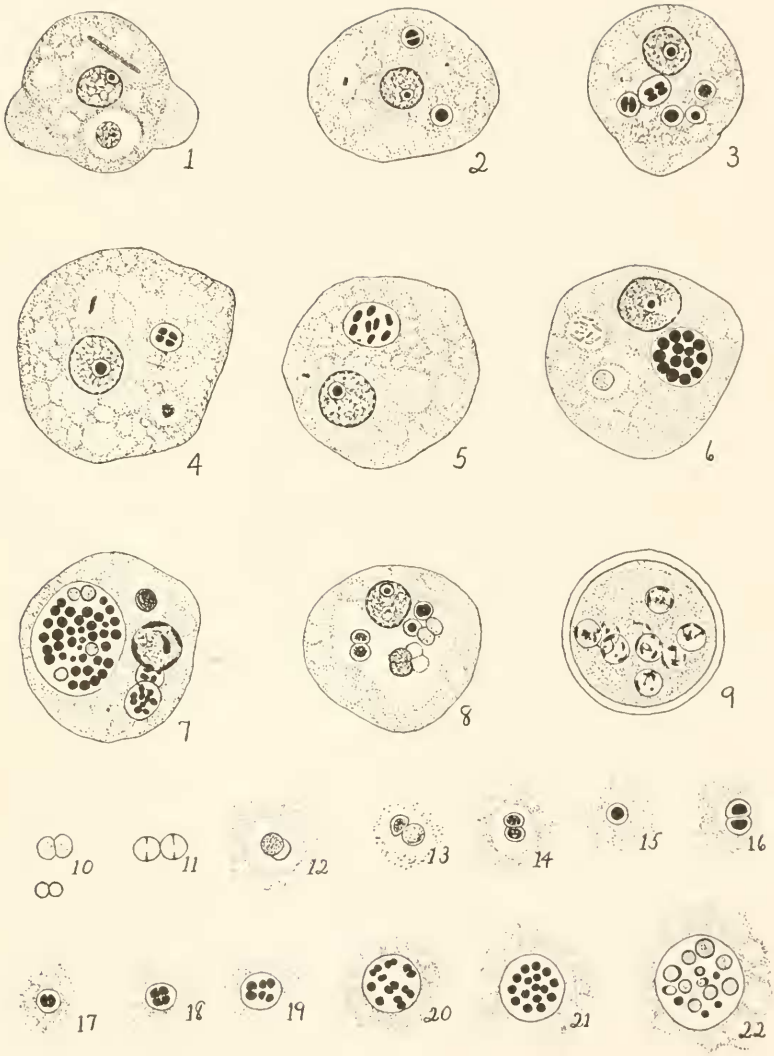
FIG. 8. Amœba with large food vacuole containing infective bacterium-like stages developing into typical uninucleate cytozoic forms.

FIG. 9. The eight-nucleate cyst of *E. citelli*.

FIGS. 10–11. Free bacterium-like stages of *Sphærita endamœbæ*.

FIGS. 12–14. Bacterium-like stages in food vacuoles of the amœba.

FIGS. 15–22. Developmental cycle of *Sphærita* in cytoplasm of amœba. Fig. 16 does not represent a division of the organism in Fig. 15, but rather the same stage of development, the next stage after Fig. 14. The two members of the pair later separate.



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MODIFICATION OF DEVELOPMENT ON THE BASIS OF DIFFERENTIAL SUSCEPTIBILITY TO RADIATION.

III. *Arbacia* Germ Cells, and (*a*) Ultraviolet Radiation, (*b*) Visible Radiation Following Sensitization.

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Experiments made with x-rays, radium, ultraviolet radiation, and visible light (following sensitization),[1] clearly demonstrate that radiation may produce an injurious effect on living tissues provided certain conditions are satisfied, namely; (*a*) the wave-length range of radiation impinging on the tissue must include that in which the tissue absorbs; (*b*) the spectral energy content must be of sufficient magnitude, and (*c*) the duration of the exposure must be long enough, to insure efficiency of action.

When intensity and duration of exposure to a given radiation, *e.g.*, ultraviolet radiation, are so regulated as to produce almost immediate death, it is found that there is an antero-posterior gradient of death and disintegration coincident (in lower animals and early embryonic stages) with the main body axis. Those regions of the body which are physiologically the most active are the first to die and disintegrate. In other words, there is a differential susceptibility to radiation in living organisms. (See also Bovie and Barr, '24, Hinrichs, '24, and Child and Deviney, '26.)

On the other hand, a sublethal dose of radiation applied in early embryonic stages, will differentially modify the later development. Here again, the regions of high physiological activity are first to be modified, and may be permanently inhibited, or if the injurious action be only slight or transitory, these regions may show recovery or acclimation more rapidly than other regions. By regulating the dosage, differential inhibition or differential acclimation (or recovery) may be obtained.

[1] See Clark, '22, Colwell and Russ, '24, Dubois, '14, Ellis and Wells, '25, Hausmann, '23, Hinrichs, '25, '26, L. Loeb, '22, for review of literature.

Such experiments have been made with (*a*) *Fundulus* and ultraviolet radiation, Hinrichs, '25, (*b*) *Arbacia* and ultraviolet radiation, Child, '24, footnote, p. 109, Hinrichs, '26, and (*c*) *Arbacia* and visible radiation following sensitization with eosin, neutral red, benzoflavine, and methylene blue, Hinrichs, '26. So far, experiments with radiation have given results entirely parallel with those produced by chemical and other physical agents, thereby further confirming the view of the non-specific and quantitative nature of susceptibility relations along the body axis.

In previous experiments it was found that the larval development of *Arbacia* may be modified by subjecting the fertilized egg, preferably during the first few minutes after insemination, to doses of radiation which, although not immediately lethal, may produce sufficient injury to interfere with the normal succession of developmental processes. In further experiments with *Arbacia*, the same types of differential modification were produced by subjecting either component of the zygote, before fertilization, to the proper dosage of radiation.

Much experimental work has been reported in which chemical treatment of either or both sex cells before insemination has brought about abnormal development. Stockard, in a series of papers, reports the production of abnormalities through the mating of alcoholized guinea pigs. (See Stockard and Papanicolaou, '18, for references to previous papers.) Rondeau and Luzeau, '01, with isotonic NaCl and sugar solutions on the eggs of *Rana fusca*, Gee, '16, with alcohol and NaOH on the germ cells of *Fundulus*, and Dungay, '13, with distilled water, chemicals, heat and cold, on *Nereis* and *Arbacia* sperm, were in each case able to produce modification of larval development; and the latter author recorded distinct differential effects. Bohn and Drzewina, '23, were able to diminish motility in the sperm of *Strongylocentrotus* by the combined action of neutral red and light, and also by the use of KCN, KCl, and of distilled water (Drzewina and Bohn, '12, '23, *a*, *b*). O. Hertwig, '13, using chloral hydrate and strychnine nitrate on the sperm of *Rana fusca*, was able to produce spina bifida, and a general delay of organ-formation and hatching. He points out the similarity

of the effects resulting from the separate treatment of either eggs or sperm. (See also G. and P. Hertwig, '13.) The latter authors, by exposing the sperm of frogs, fish, and sea urchins, to the action of chloral hydrate, strychnine nitrate, and methylene blue, were able to obtain delayed cleavage and modified development. They report, as a result of fertilizing the eggs of *Rana esculenta* by sperm of *Rana fusca*, previously treated with chloral hydrate, the appearance of short, œdematous larvæ with bent tails (and some with spina bifida) and poorly developed eyes. They further state that sperm may be injured without impairment of motility and that the sperm chromatin, although injured, remains in the egg during cleavage. This fact is further substantiated in their work on *Strongylocentrotus lividus* and *Sphærechinus granularis;* they fail to find evidence of the induced parthenogenesis which others have described.

A number of experimenters have exposed one or the other sex component to the action of radium or x-rays. Bardeen, '07, '09, modified Amphibian development by exposing sperm and eggs to radium and x-rays. His results indicate a modification of development of those body regions where growth and complex differentiation are normally most rapid, *e.g.*, neural tube, eyes, nose, and heart, *i.e.*, differential inhibition. Oppermann, '13, studied the effects of radium radiation on sperm as well as on fertilized eggs of trout. He records defects of the eyes and head and abnormal development of the tail region. G. Hertwig, '11, '12, '13, found that prolonged exposure of the sperm of frogs and *Arbacia* to radiation injures the chromatin so that it cannot take part in cell division. When unfertilized frogs' eggs are subjected to radium treatment and are then fertilized by normal sperm, the developing larvæ show the greatest modification in the developing sense organs, muscle plates, and blood cells. O. Hertwig, '11, '13, using radium on the germ cells of the frog, and the eggs of *Triton* was able to obtain differentially modified forms. P. Hertwig, '11, found that the chromatin in radium-treated *Ascaris* eggs was affected before the cytoplasm, and that cleavage was delayed and irregular and death appeared early. She also studied the behavior of the chromatin of radium-treated frog sperm, '13, and found that the sperm nucleus took no part

in the formation of the zygote nucleus nor in subsequent cleavage figures. Packard, '18, found that radiation of the egg of *Chætopterus* before fertilization prevents the egg nucleus from taking part in division, although the sperm nucleus behaves normally. Richards and Good, '19, found that radiation of *Cumingia* sperm did not affect the rate of cleavage, and that radiation of fertilized eggs produced first an acceleration in the rate of cleavage followed by a retardation. On the other hand, brief radiation of the unfertilized egg produced retarded cleavage and abnormal development. Exposure of sperm was less effective than exposure of unfertilized eggs. Redfield and Bright, '21, have shown that exposure of *Nereis* eggs to ultraviolet radiation interferes with the production of normal membranes.

In some of the above-cited work where the later development was observed, it is interesting to note that the departure from normal development appeared in systems and organs which are known to have a high rate of physiological activity. Similar conditions are found when either or both of the germ cells of *Arbacia* are exposed to ultraviolet radiation, or to visible radiation following sensitization. The region of the egg which is destined to give rise to the oral lobe and the anterior end of the pluteus has its normal development modified to a greater degree than the other parts of the egg. The resulting embryos show greater inhibition or even absence of development of the oral lobe and aboral arms.

Method.—As in previous work, two regions of the spectrum were used, the ultraviolet region, and the visible region following sensitization.

Ultraviolet Radiation was obtained from a quartz Hg-vapor arc (Cooper Hewitt), running at 60 volts, 4.0 amps. The experimental material, in open dishes, was placed approximately 30 cm. below the center of the arc. The above source is rich in ultraviolet radiation, although its spectrum extends well into the visible region (1,850–7,700 Å.).

Visible Radiation.—In this group of experiments, eggs or sperm were exposed in weak concentrations of dyes (1/2,000–1/20,000), to radiation from one of the following light sources;

1. A 1,500-watt Tungsten filament lamp burning tip-down at a

distance of 25–50 cm. above the experimental material. In some experiments, a second lamp burning tip-up was simultaneously used at the same distance below the experimental material. Temperature was controlled by the addition of ice to the water-bath surrounding the dishes. A glass dish containing a one-inch layer of water filtered out the injurious ultraviolet and heat rays.

2. A 100-watt condensed-filament lamp (of the type used with the Bausch and Lomb euscope) running at 6 volts, was placed at a distance of 25 cm. from the substage mirror of a microscope, with a water filter interposed between the lamp and the mirror. The dish containing the experimental material was placed on the microscope stage and samples of eggs or sperm were withdrawn at intervals from the center of the region reached by the light reflected up through the condenser and dish.

3. In some experiments a carbon arc, water filter, and glass lens were used in place of the above arrangement. Samples were withdrawn from the lighted portion at the focal point of the lens.

4. Direct sunlight (on mid-August afternoons) filtered through a one-inch layer of water in a glass dish was used in another set of experiments. A water bath surrounding the experimental dishes further controlled the temperature.

5. Diffuse daylight near a north window was used in another series. The dishes were covered with glass plates.

In all experiments, eggs or sperm were exposed in a thin layer just covering the bottom of the dish (about 2 mm. deep). The maximum effective periods of exposure and the intervals between the removal of successive samples were necessarily shorter with the intense radiation of the artificial light sources, than with direct sunlight or diffuse daylight. Typically, eggs fertilized by sperm which had been exposed for 45 minutes to direct sunlight showed merely a differential acclimation following a slight inhibition; while when sperm was used which had been exposed for only 5 minutes (under the above conditions) to radiation from Tungsten bulbs or the carbon arc, the result was differential inhibition with no recovery in a large proportion of the embryos.

In the experiments with visible radiation, stained and unstained sperm (or eggs) were radiated, and a parallel unradiated

control series was kept in the dark. There were further controls as follows; (*a*) in the light (in diffuse daylight throughout the experiment), and (*b*) in the dark, of each of the following: normal eggs fertilized by normal sperm (stained and unstained), unfertilized eggs (stained and unstained). This gave a check on accidental fertilization during preparation of the material, also on any possible injurious effect of the dyes. The norm for the series was furnished by the embryos resulting from the fertilization of normal eggs by normal sperm.

The results obtained with visible radiation following sensitization, and with ultraviolet radiation were essentially similar, except that the length of the effective periods of exposure to ultraviolet radiation was always much shorter; for example, differential inhibition without recovery could be obtained by exposing sperm (1/3,600 per cent.) for 10–15 seconds.

Results.—Since exposure of either sex component (before fertilization) to radiation from any one of the various light sources produces differential modification of development, and since the degree of modification is in general proportional to the dosage of radiation, it seems best to consider the results obtained all together, and to describe first the types obtained, and later the conditions under which one type or the other predominated.

The relative effectiveness of the various light sources may be determined by noting the duration of exposure required in each case to produce a given proportion of differentially inhibited forms.

A. Differential Inhibition.—Long-continued or very strong radiation of eggs (fertilized or unfertilized), and of sperm before using in the fertilization of normal eggs, produces embryos which show marked evidences of permanent differential inhibition. They are characterized by having the development of the oral lobe, aboral arms, and the region between them, more or less impaired or even entirely suppressed (Figs. 3–24).[1] Inhibition of development of the median anterior region results in the production of plutei in which the aboral arms are closer together than normal (Fig. 4), closely parallel (Figs. 5–7), or even partially

[1] Figures were drawn with the aid of a Bausch and Lomb euscope, and represent an approximate magnification of 48 diameters, following reduction.

or completely fused in the median line (Figs. 8–16). In these experiments, such forms have been obtained by exposing sperm in relatively great dilutions (1/300 per cent. to 1/6,000 per cent.), as well as with more concentrated sperm ($\frac{1}{3}$ per cent.), such radiated sperm being then used to fertilize normal eggs. The proportion of differentially inhibited forms for a given dosage is greater when the more dilute sperm is used. The same type of result, *i.e.*, differential inhibition, appears when eggs are radiated before fertilization with normal sperm. In such cases, however, under similar conditions of radiation, the percentage of permanently inhibited forms and delayed cleavage is considerably less than in the case of radiated sperm. Radiation of either sex component before fertilization, delays and often interferes with normal cleavage so that gastrulation is physically impossible. (See Table I.)

TABLE I.

EFFECT OF RADIATION ON CLEAVAGE.

A. Sperm—Visible Radiation.					*B*. Eggs and Sperm—Ultraviolet.			
Time Exposed in Min.	Eosin.	Benzo.	N. Red.	M. Blue.	Time Exposed in Sec.	1/3,600% Sperm.	Time Exposed in Sec.	Eggs.
0	100[1]	100	100	100	0	100	0	100
3	20	100	8	75	2	50	5	100
5	8	100	0	50	6	10	15	98
10	0	90	0	5	10	1	45	75

In many cases exogastrulæ are formed (Figs. 17–19). Frequently inhibition is carried to the point where the characteristics of normal plutei are completely obliterated, and the resulting larva appears as a spherical, apolar mass with non-directive swimming, with or without short skeletal arms imbedded in the tissue (Figs. 20–24).

When sperm are exposed for one and a half minutes to ultraviolet radiation, in the case of dilute sperm, or for two and a half minutes, in the case of concentrated sperm, the eggs show 80 to 100 per cent. of delayed cleavage, followed by the production of differentially inhibited larvæ. (See Table II.)

[1] Figures indicate the percentages of developing eggs which have reached the early blastula stage. The light source was a 1,500-watt bulb, at a distance of 25 cm. Sensitizing dyes were used in 1/2,000 concentration.

TABLE II.

EFFECT OF ULTRAVIOLET RADIATION OF SPERM ON DEVELOPMENT.

	A. Dilute Sperm.				*B*. Concentrated Sperm.			
Time Exposed in Sec.	N.	Accl.	Inh.	D.	N.	Accl.	Inh.	D.
30	90[1]	5	5	0	80	15	5	0
60	60	20	20	0	60	25	15	0
90	40	20	40	0	40	30	30	0
120	0	30	50	20	10	20	70	0
150	0	20	20	60	5	10	80	5
180	0	2	8	90	2	10	78	10

When eggs are exposed to ultraviolet radiation for 1 to 4 minutes, and are then fertilized by normal sperm, typically 60–80 per cent. of the resulting larvæ are normal, 10–20 per cent. show indications of recovery, about 10 per cent. are distinctly inhibited, and about 10 per cent. are dead. When the exposures exceed 5 minutes, the mortality rises to about 70 per cent., with an even distribution of normal, inhibited, and recovered forms. Long exposures interfere so seriously with cleavage that death results in early stages. The controls may be in the blastula stage, while the radiated eggs show irregular 2- and 4-cell stages.

B. Differential Acclimation and Differential Recovery.—Forms showing differential acclimation, where exposures of sperm have been slight enough to permit adjustment of the system (*i.e.*, of normal egg × radiated sperm) to new developmental conditions, and forms showing differential recovery, where inhibition due to radiation of either egg or sperm is merely transitory, show the opposite type of development from those which are permanently differentially inhibited. There may be merely an overdeveloped oral lobe, with aboral arms normal (Figs. 25–26), or the latter may appear smaller than normal (Fig. 27), or there may also be evidence of regulation in the median anterior region, as indicated by the spreading of the aboral arms (Figs. 27–28). Sometimes the angle between the arms reaches 180° (Figs. 29–30).

It is possible to obtain a good percentage of differential acclimation by fertilizing normal eggs with sperm which have

[1] Figures indicate the percentages of developing plutei which are normal (N.) acclimated (Accl.), inhibited (Inh.), or dead (D.).

had either a short exposure to high intensities, or a longer exposure to low intensities of radiation,—as for example, with sperm exposed for 45 to 60 minutes in diffuse daylight. Differential recovery may be produced by radiating eggs intensely for a short period, or less intensely for a longer period (Figs. 31–38).

Eggs stained with either of the four dyes (1/5,000) and exposed at a distance of 50 cm. for 15 minutes to radiation from a 1,500-watt lamp typically showed evidence of slight recovery in a large proportion of cases. As a rule, with exposures of 30 minutes or longer, only about 20 per cent. of the eggs remained alive, and of these a certain proportion (presumably the shaded minority) formed normal plutei. Only a small proportion of the plutei showed evidence of differential recovery after an exposure of 45 minutes. When unfertilized eggs are radiated as above, and are then fertilized by normal sperm, the proportion of abnormal larvæ which develop is smaller than in the case of eggs which are fertilized before they are radiated.

In this series, the photodynamic action of benzoflavine and eosin was less pronounced than that of neutral red and methylene blue.

Differential Inhibition Followed by Differential Acclimation or Recovery.—In experiments with radiation, particularly with ultra-violet radiation, where exposures are exceedingly short (a few seconds to 2 minutes), it is possible to have a primary inhibition followed by a secondary acclimation (Figs. 39–45) or recovery (Figs. 50–52). The oral lobe region recovers more rapidly than do other regions. In any case, the form of the larva indicates that there has been a relatively more rapid return or approach to the normal rate of physiological activity and growth of these highly susceptible regions as compared with less susceptible ones. Consequently the gradient is steepened, and the more apical regions, particularly the oral lobe, are over-developed in relation to the more basal regions, which are under-developed.

Occasionally forms appear in which more than the normal number of aboral arms are present (Figs. 46–49). Such an over-development of the skeleton is probably a result of differential inhibition persisting after general recovery. (See Child, '16, p. 115, and Hinrichs, '26.)

Discussion.—The differential modification of development produced in *Arbacia* eggs by radiation resembles so closely that produced by chemicals and other agents (Child, '16, '24, MacArthur, '24, Hinrichs, '26), that a lengthy discussion appears unnecessary here. Since the effect is differential, we may infer that a difference in susceptibility relations is established at an early stage along the axis of the egg. The results are the same whether the egg is fertilized or unfertilized at the time of radiation. Those regions of the egg which have the highest rates of physiological activity and are consequently the most intimately dependent on the continuance of normal conditions, or as R. S. Lillie puts it ('23, p. 39), on a proper "coöperation of external and internal factors," are naturally the first to show failure of equilibrium or incompatibility in their relation to their environment. (See also L. Loeb, '22.) The result is an interference, temporary or permanent, with the normal development of these regions, the degree of interference for a given dosage of radiation being in general proportional to the intimacy of dependence of the region on its environment. Such regions of high activity are also the first to acclimate or recover, provided the degree of inhibition has not been such as to produce permanent injury.

In the cases where normal eggs are fertilized by radiated sperm, the injurious action of the radiation on the sperm prevents normal development of the egg. Whether this is due to the bringing in of the toxic sperm protoplasm alone, which may be conceived of as acting in the same manner as other agents which interfere with normal development, or whether the abnormal development is an expression of incomplete fertilization bordering on parthenogenesis, as suggested by P. Hertwig, '13, is an open question which these experiments have raised but not answered. (In this connection, see also Lillie and Baskervill, '22*a*, F. R. Lillie, '11, '12, Oppermann, '13, and Dungay, '13.) I am inclined to believe that the toxic action of the radiated sperm protoplasm (after its incorporation with the egg protoplasm to form the zygote), induces abnormal development of the zygote in the same manner as do other toxic agents. There seems to be a certain analogy between this type of abnormal development and that resulting from hybridization (Newman, '17).

These experiments with sperm add further evidence for the non-specificity of susceptibility relations along the egg axis, since the characteristic difference in susceptibility is shown when the radiation acts directly on the egg protoplasm as well as when radiated sperm is used in place of normal sperm to fertilize the normal egg. On the one hand, there is a differential susceptibility to radiation, and on the other, to the action of radiated sperm. The response on the part of the egg protoplasm is the same in both cases, a differential inhibition, recovery, or acclimation. Although we find a difference in the degree of susceptibility to a given dosage of radiation, when we compare the fertilized egg, the unfertilized egg, and the sperm, in general we find that the resting unfertilized egg is less susceptible to a given dosage than the sperm before union with the egg, or than the egg when exposed immediately after union with the sperm.

The action of radiation on the sperm is shown by the reduction and frequent loss of motility (see also Lillie and Baskervill, '22*a*), as well as by the interference with normal cleavage and development. When stronger doses of radiation are used, the fertilizing power is also reduced. (See later paper for fuller discussion.)

The differential effects produced by fertilizing normal eggs with stained sperm previously exposed to sunlight or to diffuse daylight are probably referable to a summation effect of "staling" superposed on injury by radiation. In such experiments the exposures often exceeded 30 minutes or even an hour, so that although the sperm was highly concentrated when exposed, there was probably a slight falling off in normal fertilizing power during that period.[1] (See F. R. Lillie, '15, for study of effect of time and dilution on fertilizing power of sperm.)

These experiments were made at Woods Hole during the summers of 1924 and 1925, and I wish to thank Dr. R. S. Lillie, under whose direction the work was done, for his interest and coöperation.

Conclusions.—From these results, further evidence is obtained for the following:

[1] G. and P. Hertwig, '13 report two-hour exposures of the sperm of *Sphærechinus* and *Strongylocentrotus* to the action of chloral hydrate, methylene blue, etc. A two-hour delay in the use of *Arbacia* sperm results in considerable loss of fertilizing power, even under normal conditions.

1. In order that visible radiation following sensitization may be effective, the sensitized system, in this case eggs or sperm before fertilization, must be exposed to radiation of sufficient intensity and duration whose wave-length range includes that absorbed by the particular sensitizer used.

2. Ultraviolet radiation is effective without the aid of a sensitizer, by virtue of its direct absorption by protoplasm.

3. Susceptibility to sublethal doses of radiation in these two spectral regions is a differential one, *i.e.*, regions of high physiological activity are the first to be modified in their development. They are also the first to recover when the injurious effect is slight.

4. Modification of development by means of radiation produces results essentially similar to those obtained by other means.

5. Development may be modified by subjecting fertilized eggs, soon after insemination, to the action of intense radiation as in previously reported experiments. Modification of development may also be obtained by exposure of either sex component of the zygote to intense radiation previously to fertilization.

6. A differentially modified larva resulting from the union of radiated eggs and normal sperm is evidence that differences in the susceptibility of the various regions already exists in the unfertilized egg.

7. A differentially modified larva developing from a zygote the sperm component of which has been injured by radiation may be either differentially inhibited or differentially acclimated.

8. Since the developing embryo becomes differentially modified, it appears that the egg at the time of fertilization already shows a difference in susceptibility of its various regions to the injurious action of the radiated sperm.

9. Radiation of sperm reduces its motility, delays cleavage, and interferes with normal development of the zygote.

10. Radiation of sperm inhibits its fertilizing power. (See later paper.)

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DESCRIPTION OF FIGURES.

PLATE I. FIGS. 1–30.

Normal Larvæ.—Figs. 1–2. Fig. 1, aboral, and Fig. 2, lateral view of normal pluteus, 48 hours after fertilization.

Differential Inhibition.—Figs. 3–24.

FIG. 3. Eggs exposed 45 minutes in 1/5,000 Eosin. 1,500-watt bulb, at 50 cm.

FIG. 4. Eggs exposed 15 minutes in 1/5,000 N. red. 1,500-watt bulb, at 50 cm.

FIG. 5. Unstained sperm 10 minutes. 1,500-watt bulb, at 25 cm.

FIG. 6. Concentrated sperm 1 minute. Ultraviolet.

FIGS. 7–8. Eggs exposed 45 minutes in 1/5,000 N. red. 1,500-watt bulb, at 50 cm.

FIG. 9. Sperm exposed 10 minutes, natural pigment. 1,500-watt bulb, at 50 cm.

FIGS. 10–11. Sperm (dilute) $2\frac{1}{2}$ minutes. Ultraviolet.

FIG. 12. Sperm (conc.) 2 minutes. Ultraviolet.

FIG. 13. Sperm exposed 30 minutes, natural pigment. 1,500-watt bulb, at 50 cm.

FIG. 14. Sperm exposed 30 minutes in 1/10,000 Eosin. 1,500-watt bulb, at 50 cm.

FIG. 15. Eggs exposed 45 seconds. Ultraviolet.

FIG. 16. Eggs exposed 45 minutes in 1/5,000 N. red. 1,500-watt bulb, at 50 cm.

FIG. 17. Eggs exposed 15 minutes in 1/5,000 Benzo. 1,500-watt bulb, at 50 cm.

FIGS. 18–19. Sperm exposed 30 minutes in 1/10,000 Eosin. 1,500-watt bulb, at 50 cm.

FIG. 20. Sperm (conc.) 3 minutes. Ultraviolet.

FIG. 21. Sperm exposed 2 seconds. Ultraviolet.

FIG. 22. Sperm exposed 30 minutes in 1/10,000 Eosin. 1,500-watt bulb, at 50 cm.

FIG. 23. Sperm (conc.) 2 minutes. Ultraviolet.

FIG. 24. Sperm (conc.) $2\frac{1}{2}$ minutes. Ultraviolet.

Differential Acclimation.—Figs. 25–30.

FIG. 25. Sperm (dil.) 1 minute. Ultraviolet.

FIG. 26. Sperm (conc.) $\frac{1}{2}$ minute. Ultraviolet.

FIG. 27. Sperm 10 minutes, natural pigment. 1,500-watt bulb, at 50 cm.

FIG. 28. Sperm (conc.) 1 minute. Ultraviolet.

FIG. 29. Sperm 10 minutes, unstained. 1,500-watt bulb, at 25 cm.

FIG. 30. Sperm 10 minutes in 1/10,000 Eosin. 1,500-watt bulb, at 25 cm.

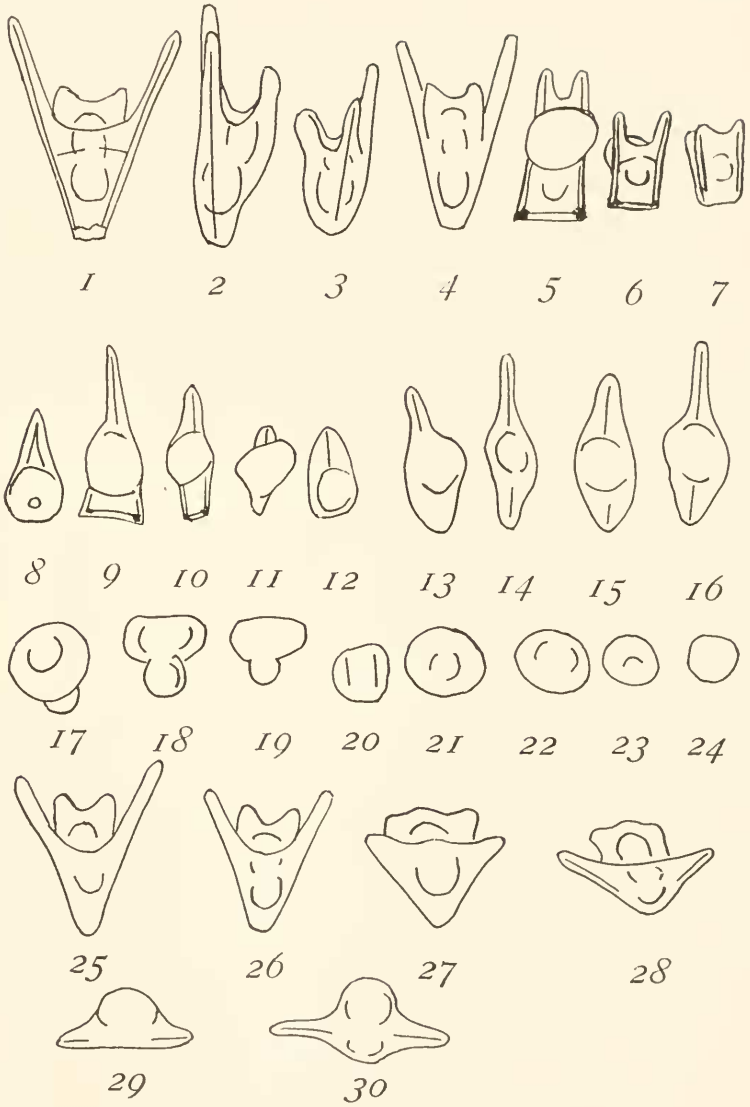

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PLATE II. FIGS. 31–52.

Differential Recovery.—Figs. 31–38.

FIG. 31. Eggs exposed 15 minutes in 1/5,000 Benzo. 1,500-watt bulb, at 50 cm.

FIG. 32. Eggs exposed 45 minutes in 1/5,000 M. blue. 1,500-watt bulb, at 50 cm.

FIG. 33. Eggs exposed 15 minutes in 1/5,000 Benzo. 1,500-watt bulb, at 50 cm.

FIG. 34. Eggs exposed 45 minutes in 1/5,000 M. blue. 1,500-watt bulb, at 50 cm.

FIG. 35. Eggs exposed 15 minutes in 1/5,000 Benzo. 1,500-watt bulb, at 50 cm.

FIG. 36. Eggs exposed 45 minutes in 1/5,000 Benzo. 1,500-watt bulb, at 50 cm.

FIG. 37. Eggs exposed 45 minutes in 1/5,000 N. red. 1,500-watt bulb, at 50 cm.

FIG. 38. Eggs exposed 15 minutes in 1/5,000 N. red. 1,500-watt bulb, at 50 cm.

Differential Inhibition Followed by Acclimation.—Figs. 39–49.

FIG. 39. Sperm (conc.) $\frac{1}{2}$ minute. Ultraviolet.

FIG. 40. Sperm (dil.) 3 minutes. Ultraviolet.

FIG. 41. Sperm (conc.) 3 minutes. Ultraviolet.

FIG. 42. Sperm 10 minutes in 1/10,000 Eosin. 1,500-watt bulb, at 50 cm.

FIG. 43. Sperm 30 minutes unstained. 1,500-watt bulb, at 25 cm.

FIG. 44. Sperm (conc.) $\frac{1}{2}$ minute. Ultraviolet.

FIG. 45. Sperm (dil.) $2\frac{1}{2}$ minutes. Ultraviolet.

FIG. 46. Sperm (conc.) 1 minute. Ultraviolet.

FIG. 47. Sperm 10 minutes in 1/10,000 Eosin. 1,500-watt bulb, at 50 cm.

FIG. 48. Sperm 30 minutes in 1/10,000 Eosin. 1,500-watt bulb, at 50 cm.

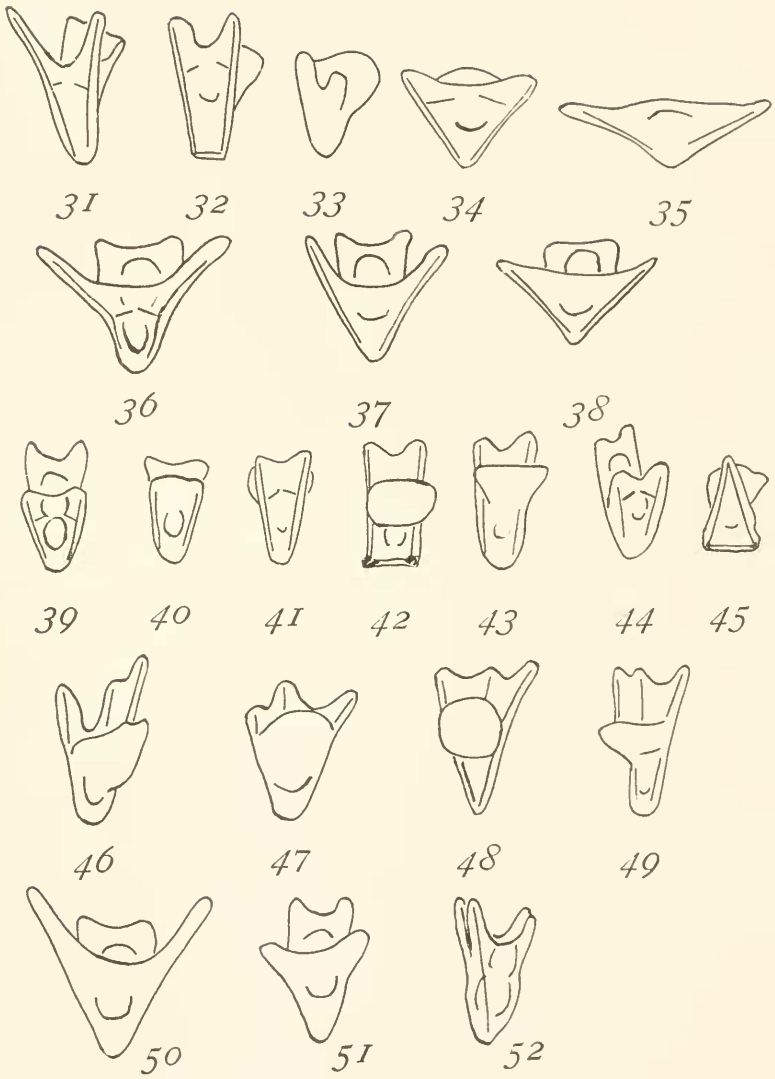
FIG. 49. Sperm 30 minutes unstained. 1,500-watt bulb, at 25 cm.

Differential Inhibition Followed by Recovery.—Figs. 50–52.

FIG. 50. Eggs exposed 15 minutes in 1/5,000 Benzo. 1,500-watt bulb, at 50 cm.

FIG. 51. Eggs exposed 45 minutes in 1/5,000 Benzo. 1,500-watt bulb, at 50 cm.

FIG. 52. Eggs exposed 45 minutes in 1/5,000 Benzo. 1,500-watt bulb, at 50 cm.



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THE EFFECT OF ULTRAVIOLET RADIATION ON THE FERTILIZING POWER OF *ARBACIA* SPERM.

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It is a well established fact that ultraviolet radiation may have an injurious effect on living tissues under certain conditions. That *Arbacia* sperm is no exception is evident from the diminution of fertilizing power following exposure of sperm to radiation from a quartz mercury-vapor arc whose spectrum range covers approximately 1,850–7,700 Å.

A number of chemical and physical agents have been used to injure the sperm of various animals. In general, such treatment results in a decrease of motility and a lowering of fertilizing power. In eggs which have been fertilized by treated sperm, cleavage is delayed and irregular, gastrulation often impossible, and development abnormal, particularly in the regions of relatively high physiological activity.[1]

Sperm may be considered as being endowed with a definite and limited amount of available energy which is expended at a ratio determined by its activity. Cohn, '18, found that the total amount of carbon dioxide produced during the lifetime of sperm was constant; the more active the sperm, the shorter its life. The same investigator (Cohn, '17) found that sperm in acidified water retained their fertilizing power for a long time, although they remained relatively inactive.

Certain chemical agents are known to stimulate sperm to greater activity, *e.g.*, alkalies, thereby decreasing the length of life of the sperm. (See Gray, '15, and Gee, '16.) Certain investigators have found that eggs give off substances which stimulate sperm to greater activity. Fuchs, '14, found that the blood of freshly caught animals increased the fertilizing power of certain sperm. The same author, in a study of cross-fertilization

[1] For a review of the literature in this connection, see previous paper, Hinrichs, '26*b*.

(Fuchs, '15), found that some egg secretions may produce an increase in the fertilizing power of the sperm of another species. Clowes and Bachman, '20, have obtained a volatile sperm-stimulating substance from *Arbacia* eggs.

On the other hand, a number of agents have been used to inhibit the activity of sperm, for example, neutral red in the presence of light, ultraviolet radiation, radium, temperature, x-rays, the electric current, and various chemicals.[1] (See Bohn and Drzewina, '23*b*, Lillie and Baskervill, '22, Oppermann, '13, Dungay, '13, Bardeen, '07, Günther, '07, Cohn, '17, Gee, '16, and others for references.) KCN, which interferes with normal oxidation processes, was found by Drzewina and Bohn, '12, to inhibit the activity of sea urchin sperm as well as to cause irregular cleavage and abnormal development of eggs fertilized by the treated sperm. The ageing of sea urchin germ cells has been found by Goldfarb, '17, '18, to proceed more rapidly after their removal from the body. Correlatively motility and fertilizing power are reduced. Hyperalkaline sea water accelerates the ageing process.

Time and dilution are both known to be factors affecting the fertilizing power of *Arbacia* sperm (F. R. Lillie, '15*b*). Drzewina and Bohn, '23*b*, also showed dilution to be a factor in the susceptibility of sea urchin sperm to the combined action of neutral red and light. Motility and fertilizing power were lost more quickly in dilute than in concentrated sperm suspensions. Usually loss of motility is associated with loss of fertilizing power, but the two do not exactly parallel each other. Fertilizing power is not a function of motility alone, and declines more rapidly than does motility. (See F. R. Lillie, '15*b*, and Lillie and Just, '24.) Sperm may be injured in such a way that its fertilizing power, as measured by the proportion of eggs fertilized and the normality of the cleavage and development, is materially lessened while motility is not visibly impaired. (See also Hinrichs, '26*b*, and Lillie and Baskervill, '22.)

[1] It has been repeatedly pointed out, especially by O., G., and P. Hertwig, (see previous paper for references) that the effect of injury on the sperm is particularly felt by the nuclear material. Packard, '14, however, suggests also a possible effect on the cytoplasm. He postulates the presence of enzymes which may be injured by radiation.

Method.—In these experiments, measured dilutions of sperm (1 per cent. to 1/240 per cent.) were exposed, as a thin film just covering the bottom of the dish, to radiation from a Cooper Hewitt quartz mercury-vapor arc at a distance of 30 cm. for one minute. (Temperature was controlled by means of a water bath. No attempt was made to screen out visible or other rays from the spectrum of the arc.) Dilutions of sperm were made after the manner of F. R. Lillie, '15*b*, on a percentage basis, using as "stock" the thick fresh sperm as it exuded from the genital pore of cut, inverted *Arbacia* males.

Immediately after exposure, samples of one drop each of radiated and nonradiated sperm of the same dilution, were transferred to dishes containing 10 cc. (300 drops) [1] of a known concentration of eggs in sea water (3–5 per cent. in these experiments). In drawing off samples of radiated sperm, care was taken to obtain sperm from the upper surface at the center of the dish. At short intervals thereafter, up to three hours, samples of radiated and nonradiated sperm, kept under similar conditions, were used to fertilize normal eggs. At the end of 4 or 5 hours, the percentage of eggs which had formed membranes and of those which had cleaved was determined on the basis of a count of 200 eggs in each case.

By comparing results obtained following ultraviolet radiation, with those obtained from normal lots of eggs it was possible to estimate the degree to which radiation had inhibited the fertilizing power of a given dilution of sperm.

Results.—It was found that in normal sperm suspensions, fertilizing power decreased as time after removal of the first sample increased; the more dilute the sperm suspension, the more rapid was the rate of decrease. (See also F. R. Lillie, '15*b*.) Time and dilution, then, are factors in determining the fertilizing power of normal sperm. (See Table I.)

Exposure of dilute sperm to ultraviolet radiation augments the rate of loss of fertilizing power beyond that following dilution alone. Fewer eggs cleave (see Table I.), or even form mem-

[1] Sperm was thus further diluted 300 times. Concentrations of sperm at the time of insemination were therefore 1/300 per cent. to 1/72,000 per cent., ($\frac{1}{2}^8$ per cent. to $\frac{1}{2}^{16}$ per cent.)

Table I.

The Effect of Radiation on the Fertilizing Power of Sperm.[1]

Time (minutes).	*A.* Normal Nonradiated Sperm.									*B.* Radiated Sperm.								
	Dilution of Sperm (Powers of 2).																	
	8	9	10	11	12	13	14	15	16	8	9	10	11	12	13	14	15	16
	Percentage of Eggs Cleaved.									Percentage of Eggs Cleaved.								
0	100	99	100	100	99	90	95	73	36	97	88	91	68	49	24	22	8	2
3	100	—	98	—	—	76	—	—	14	92	—	84	—	—	11	—	—	0
5	100	99	98	100	98	73	94	54	5	97	96	72	55	36	7	8	6	.5
10	100	—	—	98	—	—	89	51	7	99	—	—	49	—	—	5	4	0
15	100	99	98	99	95	49	67	46	6	99	84	44	51	19	2	5	3	0
30	97	100	92	94	68	18	30	41	1	95	96	21	40	13	2	4	1	0
45	99	100	91	70	63	27	—	14	.5	93	94	30	42	5	3	—	0	0
60	99	100	92	65	49	19	30	11	.5	43	82	21	35	2	1	1	.5	0
90	94	96	28	54	28	17	15	2	0	25	89	11	22	1	1	0	0	0
120	94	—	29	38	21	3	7	2	—	5	—	2	15	.5	1	.5	0	—
150	—	94	—	—	9	—	1	.5	—	—	25	—	—	0	—	.5	0	—
180	88	—	12	27	2	3	2	.5	0	2	—	0	6	0	1	0	0	0

branes, in comparable lots of eggs fertilized by exposed sperm, as compared with eggs fertilized by normal sperm. Also, the rate at which fertilizing power is lost is greater in the more dilute suspensions. (See Fig. 1.) Bohn and Drzewina, '23, showed that the more dilute the sperm suspensions of *Arbacia* were, the more susceptible they were to the inhibiting influence of neutral red in the presence of light.

Figure 1 indicates that in general, radiation shifts the curve of the dilution effect to the left along the time axis. The greater the dilution, the more immediately is the rate of falling off augmented as compared with normal lots. For example, Fig. 1 gives the following data with respect to increase in the rate of falling off in the fertilizing power of *Arbacia* sperm. In dilution $\frac{1}{2}^{8}$ per cent. (Curve *A*), the increase in rate is not felt until one hour after exposure, but the continued decrease in fertilizing power lasts until three hours after exposure. In more dilute

[1] Measured by the percentage of cleavage in normal eggs fertilized by treated sperm.

Figures 8–16 represent sperm dilutions of $\frac{1}{2}^{8}$–$\frac{1}{2}^{16}$%. (11 and 12 are averages of three experiments; 15 of two; and the rest, one each.)

Figures in the first column to the left represent the time in minutes following initial dilution and exposure to radiation.

suspensions, $\frac{1}{2}^{10}$ per cent., $\frac{1}{2}^{12}$ per cent. (Curve *B*), $\frac{1}{2}^{14}$ per cent., the increase in rate is noticeable only up to one hour, $\frac{3}{4}$, and $\frac{1}{4}$ hours respectively. With still greater dilution, $\frac{1}{2}^{15}$ per cent.

FIG. 1. The effect of radiation on the fertilizing power of sperm. Time and dilution are factors. The more dilute suspensions are more susceptible, particularly immediately after radiation. *A*, *B*, *C*—normal nonradiated sperm. *a*, *b*, *c*—radiated sperm in corresponding dilutions. *A*, *a*—$\frac{1}{2}^{8}$%; *B*, *b*—$\frac{1}{2}^{12}$%; *C*, *c*—$\frac{1}{2}^{15}$% sperm dilutions. Ordinates represent the percentage of eggs cleaved. Abscissæ represent the time in hours, since the beginning of the exposure of sperm to radiation.

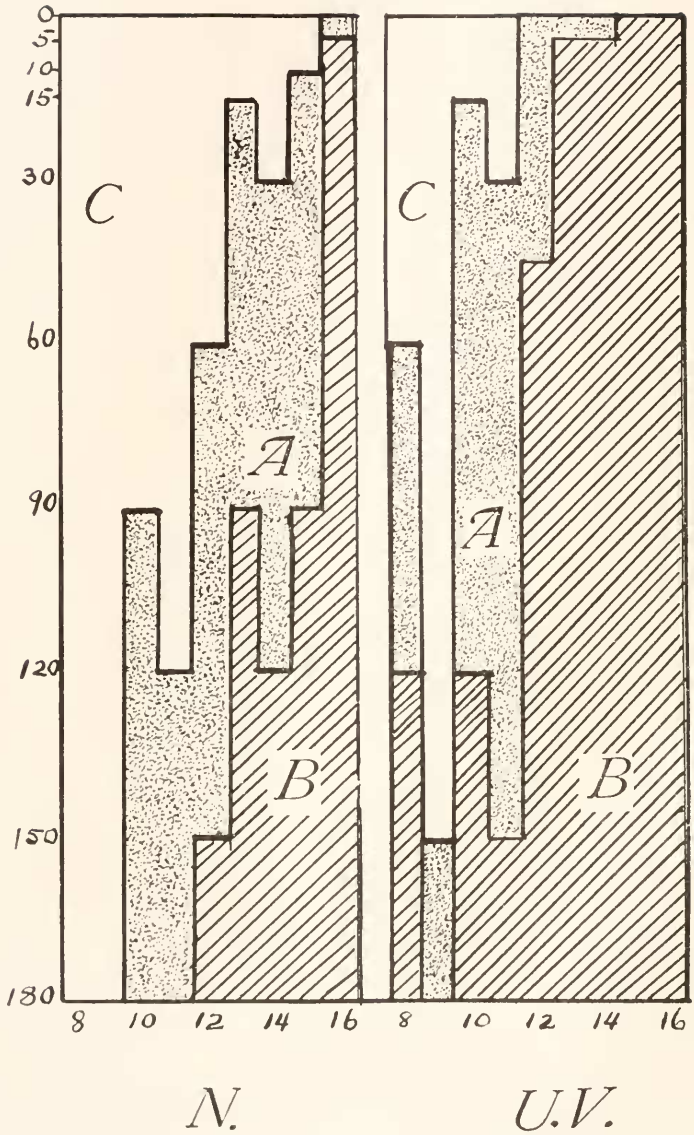

FIG. 2. Reduction of fertilizing power. Radiation reduces the time required to decrease the fertilizing power in all dilutions. Again, the more dilute suspensions are more susceptible. *N*—normal nonradiated sperm. U.V.—radiated sperm. *A*, *B*, *C*—represent percentages of eggs in early cleavage stages. *A*—a reduction of from 10 to 50% in the number of eggs which cleaved. *B*—a reduction of from 90% in the number of eggs which cleaved. *C*—a reduction of from 0 to 50% in the number of eggs which cleaved. 8–16 represent sperm dilutions, *e.g.*, $\frac{1}{8}$% to $\frac{1}{16}$%. Ordinates represent the time in minutes elapsed since the beginning of the experiment. Abscissæ represent the degree of sperm dilution.

(Curve *C*) to $\frac{1}{2}^{16}$ per cent. the increase in rate of loss of fertilizing power is immediate.

A study of Fig. 2 reveals the fact that it takes longer to reduce the fertilizing power below 50 per cent. in normal sperm suspensions than in radiated suspensions of comparable dilution; that more time is needed to bring about a 50 per cent. reduction in the less dilute suspensions; and that when normal eggs are fertilized by dilute radiated sperm ($\frac{1}{2}^{12}$ per cent. to $\frac{1}{2}^{14}$ per cent.), less than 50 per cent. cleavage follows; and finally that when sperm dilutions reach $\frac{1}{2}^{15}$ per cent. to $\frac{1}{2}^{16}$ per cent., less than 10 per cent. of the eggs cleave. (See Table II.) The figures indicate the number of minutes, following initial dilution and exposure of sperm suspensions, required to reduce cleavage 50 per cent. and 90 per cent. It will be seen that less time is required in dilute suspensions, indicating that they are more susceptible to radiation effects.

TABLE II.

THE EFFECT OF RADIATION OF SPERM ON THE PERCENTAGE OF CLEAVAGE OF NORMAL EGGS.

	A. Normal Nonradiated Sperm.									*B*. Radiated Sperm.								
	8	9	10	11	12	13	14	15	16	8	9	10	11	12	13	14	15	16
Time Required to Reduce Cleavage.[1]																		
50%	—	—	60	90	45	10	15	10	0	45	120	10	15	0	0	0	0	0
90%	—	—	—	—	120	60	90	60	5	90	120	90	150	30	10	5	0	0

That cleavage is retarded and abnormally modified by the radiation of sperm, and that the length of time elapsed after dilution determines the severity of the effect, even in non-radiated suspensions, is clearly shown. When sperm in the concentration of $\frac{1}{2}^{11}$ per cent. is radiated for 5 minutes and is then used at intervals to fertilize normal eggs, no larvæ are formed, and cleavage is abortive and reduced 90 per cent. or more. Table III. shows such a series for normal and radiated sperm in like concentrations.

[1] Time in minutes, following dilution, required to bring the sperm into a state at which 50 or 90 per cent. of the eggs fail to cleave.

Figures 8–16 represent sperm dilutions of $\frac{1}{2}^{8}$–$\frac{1}{2}^{16}$ per cent.

Table III.

The Effect of Radiation on $\frac{1}{2}^{11}$ Per Cent. Sperm when Time is Also a Factor.

(Sperm was radiated for five minutes, and immediately thereafter used to fertilize normal eggs.)

A. Normal Nonradiated Sperm.		*B*. Radiated Sperm.
Time[1]		
0	100% swimming larvæ	No larvæ; less than 1% abortive cleavage
3	100% " "	" " 1% " "
5	99% " "	" " " " 1% " "
11	100% " "	" " 2% " "
15	100% " "	" " " " 1% " "
30	97% " (3% abnormal)	" " " " 1% " "
45	80% " (10% ")	" " 3% " "
60	50% " (30% ")	" " 7% " "
90	25% " (50% ")	" " 10% " "
150	5% " (75% ")	" " 1% " "

When examined five minutes after exposure, less than 1 per cent. of the radiated sperm show any motility. Later, however, after about nine hours the sperm may be seen to cluster about the eggs, indicating some degree of recovery of motile power. The recovery of motile power following inhibition by radiation, recalls the assertion made by Glaser, '14, namely, that paralyzed sperm may be reactivated but not reagglutinated.

Motility is considerably reduced soon after exposure to ultraviolet radiation (see also Lillie and Baskervill, '22). Sperm in the more dilute suspensions more quickly lose their power of movement, and clumping or agglutinating takes place early. The clumps are large and irregular and stable. The higher the dosage, the larger are the clumps of sperm. In viewing an exposed lot of sperm with a microscope, it can be seen that the surface layer contains immobile sperm, while the layers below (as seen in optical section) have been "shaded" from the ultraviolet radiation (or have not been reached because of insufficient penetration of the rays), and are therefore normally active. The extreme bottom layer of the dish again contains inactive sperm which have probably sunk down from the top layer. Clusters of sperm are numerous. In transmitted light, radiated sperm suspensions appear "clear," while nonradiated ones appear "milky."

When sperm in dilutions ranging from 1/300 per cent. to

[1] Time elapsed (in minutes) since exposure to radiation for 5 minutes.

1/240,000 per cent. is exposed to radiation from the mercury arc for periods of 2 and 5 minutes, it becomes evident that the more dilute suspensions are more susceptible to reduction in fertilizing power than the more concentrated suspensions. Nonradiated controls show 100 per cent. cleavage in all dilutions up to 1/30,000 per cent., in which case 60 per cent. of the eggs cleave normally, 15 per cent. abnormally, and 25 per cent. fail to cleave. When a dilution of 1/240,000 per cent. is reached the total cleavage amounts to but 9 per cent., when sperm are used immediately after radiation to fertilize normal eggs. Dilution of sperm is certainly a factor in modifying its fertilizing power, even in non-radiated suspensions.

Table IV. shows the effects of radiation on cleavage. It will be seen that membrane formation is possible, with less dilute suspensions of sperm when radiation does not exceed 2 minutes. That the degree of inhibitory effect of radiation increases with dosage is indicated here.

TABLE IV.

THE EFFECT OF RADIATION OF SPERM ON THE RATE OF CLEAVAGE OF EGGS.

(Eggs were fertilized immediately following dilution and radiation of sperm.)

	A. Sperm Radiated for 2 Minutes.									*B*. Sperm Radiated for 5 Minutes.								
	Dilution of Sperm (Powers of 2).																	
	8	9	10	11	12	14	15	17	18	8	9	10	11	12	14	15	17	18
A.....	25	14	0	30	12	1	<1	—	—	5	2	—	15	<1	1	1	—	—
B.....	6	8	10	2	<1	<1	—	<1	—	1	2	2	1	—	—	—	—	—
C.....	60	3	5	1	<1	<1	—	—	—	—	1	—	—	—	—	—	—	—
D.....	9	75	85	67	87	97	99	99	100	94	95	98	84	99	99	99	100	100

Dilutions as before. 11 and 15 are averages of three experiments each; 14 of two; and the rest, one each.

A—Advanced cleavage.
B—Slow cleavage.
C—Membranes only.
D—No membranes.

That the degree of the effect of radiation is roughly proportional to dosage is better shown in Table V., where the exposures were briefer. In these experiments, on the day following radiation of sperm, swimming preplutei were found in radiated lots, only with sperm dilutions of $\frac{1}{2}^{8}$ per cent.; early gastrulæ were plentiful in $\frac{1}{2}^{9}$–$\frac{1}{2}^{14}$ per cent., and scarce in $\frac{1}{2}^{15}$–$\frac{1}{2}^{18}$ per cent. In the control series (nonradiated) all dilutions showed swimming preplutei.

The effect of ultraviolet radiation on fertilizing power is, in general, roughly proportional to the dosage, *e.g.*, exposures of 5 minutes are more effective in causing immediate reduction of fertilizing power than are exposures of a minute or less. Also,

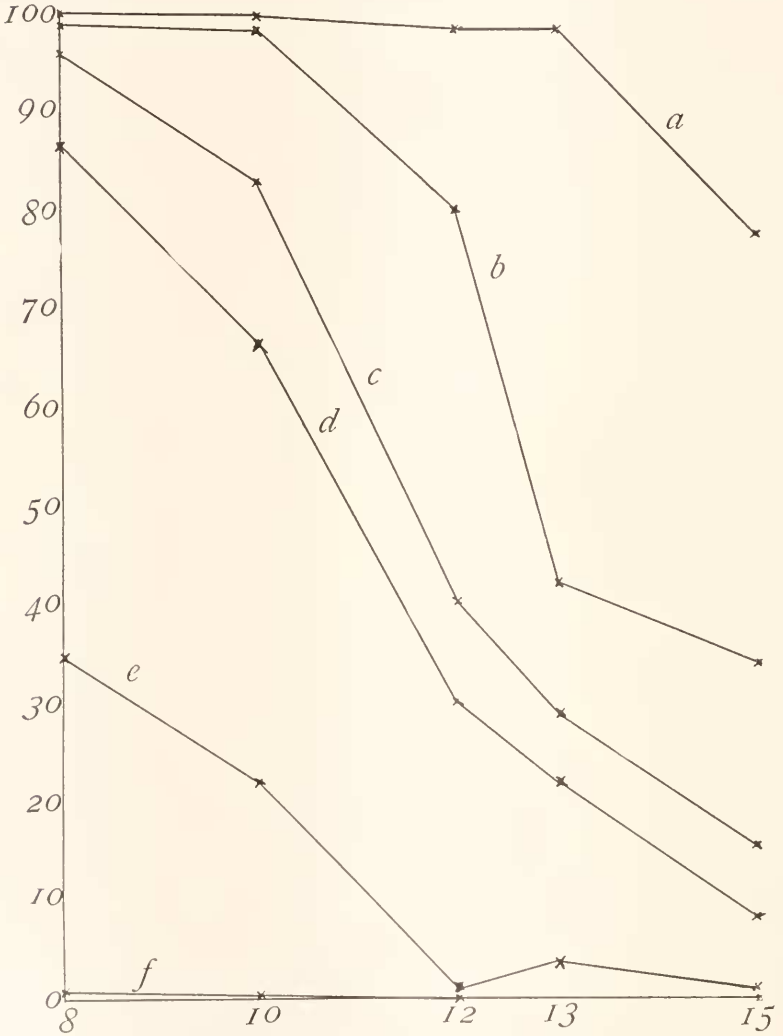

FIG. 3. The effect of length of period of exposure on fertilizing power. The degree of reduction is roughly proportional to the dosage of radiation. *a*–*f* represent the lengths of the periods of exposure in minutes. *a*—0, *b*—$\frac{1}{4}$, *c*—$\frac{1}{2}$, *d*—1, *e*—3, *f*—10. Ordinates represent the percentage of eggs cleaved. Abscissæ represent the dilutions of sperm, *e.g.*, 8—$\frac{1}{2}$[8]%, etc.

Table V.

The Relation of Dosage of Radiation to the Degree of Inhibition of Cleavage.

Dosage in min.	Dilution of Sperm (Powers of 2).				
	8	10	12	13	15
	Percentage of Eggs Cleaved.				
0	100	99.5	98	98	77
¼	99	98	80	42	34
½	96	83	40	29	15
¾	87	79	42	20	11
1	87	67	30	22	8
3	35	22	1.5	4	1.5
5	2	1	.5	1	.5
10	1	.5	0	0	0

Figures 8–15 represent sperm dilutions $\frac{1}{2}^{8}$–$\frac{1}{2}^{15}$ per cent.

the degree of reduction in each case is more marked in the weaker suspensions, *e.g.*, exposures of less than one minute reduce the fertilizing power 50 per cent. in dilutions of $\frac{1}{2}^{12}$ per cent. to $\frac{1}{2}^{15}$ per cent. The same exposure reduces the fertilizing power of dilutions of $\frac{1}{2}^{8}$ per cent. to $\frac{1}{2}^{10}$ per cent. only 10–20 per cent. Five and ten minute exposures reduce the fertilizing power to 2 per cent. or less in all dilutions. (See Figure 3.)

Discussion.—The loss of fertilizing power of normal sperm suspensions occasioned by dilution and staling is greatly accelerated by exposure to ultraviolet radiation. The greater the dilution of the sperm, the more rapid is the reduction of fertilizing power, and the more delayed and abnormal is the cleavage. Dosage of radiation is also a factor in determining the degree of inhibition of fertilizing power. Motility is reduced and the sperm forms aggregations which are large and irregular, and recall those described by F. R. Lillie, '12*b*, '13, '15*a*, Just, '19, J. Loeb, '14, and Sampson, '22. Exposure to ultraviolet radiation appears to increase the surface adhesiveness of sperm, inducing agglutination, a change probably accompanied by a change in permeability of the sperm membrane. (See also F. R. Lillie, '13, in this connection.)

Although motility is considerably interfered with, the loss of fertilizing power does not necessarily parallel the loss of motility.

(See F. R. Lillie and Just, '24, p. 134.) Exposures up to 5 minutes produce but a slight immediate inhibition of motility; exposures of 10–20 minutes produce a noticeable inhibition, while exposures of 30 minutes completely paralyze the sperm. However, even after so long an exposure, a small percentage of the eggs reach the 2- and 4-cell stage (less than 1 per cent.), but proceed no further, suggesting that normal fertilizing power is probably impaired before motility is lost. On the other hand, shorter exposures ($\frac{1}{4}$ min. to 1 min.) produce a marked reduction of fertilizing power as measured by the percentage of eggs which produce membranes and cleave.

It seems probable that the loss of fertilizing power is due rather to the outward diffusion of some substance necessary for fertilization. Such a substance, if present at or near the surface of the sperm, may easily diffuse into the medium by changes induced in the sperm surface by surface-acting radiation. This would result in the disturbance of the normal concentration equilibrium of such a substance in the sperm which would then become incapable of inducing normal and complete fertilization, since the latter probably depends on an optimum concentration of such a substance within the sperm.

As before stated, highly diluted sperm suspensions are more susceptible to injury than are the more concentrated suspensions. Diffusion of sperm substance to the external medium following a change in the permeability of the sperm surface induced by radiation, would proceed more rapidly in dilute than in concentrated suspensions, since fewer sperm per unit volume of suspension would contribute to the establishing of an external-internal equilibrium. Diffusion of a substance necessary for fertilization, from the sperm into the medium may explain the loss of fertilizing power induced by dilution and long standing. It may also explain why more dilute suspensions are more susceptible to an influence which is additive in its action on the diffusion rate of such a substance.[1]

[1] Compare the mass effects obtained by Robertson, '22, Drzewina and Bohn, '21, '22. See also a later paper by Drzewina and Bohn, '23*a*, for suggestion of mass effect on sperm. Glaser's suggestion (Glaser, '15) of the necessity for the presence of more than one sperm per egg in order to insure successful fertilization, may have some bearing on the problem of mass effects in sperm.

When fertilization is incomplete or partial, abnormal cleavage and abnormal development follow. (See Table IV; also previous paper, Hinrichs, '26*b*.) F. R. Lillie, '11, '12*a*, '19, in analyzing the fertilization reaction in *Nereis*, divided it into two phases, external and internal. The former concerns itself with membrane formation and needs only the contact of sperm for its initiation. Other investigators have obtained similar results; thus, Just, '19, found that although sperm entry may initiate cortical changes, it is no criterion of fertilization, and further development does not necessarily follow. Loeb, '13, states that cortical changes in the sea urchin are not dependent on the penetration of sperm. Dungay, '13, found that injury of sperm may prevent its entry into the egg, and maturation may go on, but cleavage fail to follow. (See also the recent paper by O. Hyman, '25.)

In the experiments with ultraviolet radiation, membrane formation is usually less interfered with than cleavage. There may be an advance of from .5–5 per cent. in the number of eggs having membranes only, over those cleaved. This seems to be an indication that the fertilization reaction was initiated but not completed.

Complete internal fertilization involves compatibility of germinal nuclear material, and forms the basis for normal development. When either sex component has been injured, *e.g.*, by ultraviolet radiation, development becomes abnormal, particularly in the regions of the organism which normally have a relatively high rate of physiological activity. (See also Hinrichs, '26*b*.) Such a result is comparable to that reported by Newman, '17, for teleost hybrids, where incomplete fertilization resulting from the incompatibility of germ plasms produces differentially modified embryos.

It would be difficult to explain so complete a series of differentially inhibited forms as those shown in Plates I. and II. of a previous paper (Hinrichs, '26*b*) on the assumption of parthenogenetic development of the egg induced by injury to the sperm nuclear material. Also, the fact that the degree of effect (as measured by the percentage of eggs cleaved and the type of cleavage) is proportional to the dosage of radiation to which the sperm is subjected, can hardly be satisfactorily explained in this way.

Cohn, '17, '18, has shown that sperm in dilute suspensions are more active than in concentrated suspensions. Active cells are, on the whole, more susceptible to injury by radiation than are quiescent cells. (See Hinrichs, '24, '26*b*.)

Ultraviolet radiation is particularly suited for experiments of this kind, as compared with chemical agents, because,

(1) Exposures may be very short and still be effective. A superposition of the effect of staling is thus impossible.

(2) The possibility of carrying over into the normal egg, at the time of fertilization, small amounts of injurious reagent is absent. Results are clear-cut and more likely to be due to ultraviolet radiation alone, uncomplicated by other factors.

I wish here to express my thanks to Dr. R. S. Lillie for his interest and help in the study of this problem. .

Conclusions.—(1) Further data are given on the normal loss of fertilizing power in sperm suspensions due to dilution and staling. These results are entirely in accord with the ones reported by F. R. Lillie in 1915.

(2) Ultraviolet radiation augments the rate of loss of fertilizing power beyond that produced by time and dilution.

(3) Dilute sperm suspensions lose their power of fertilization earlier and more rapidly when radiated (and even without radiation) than do the more concentrated suspensions.

(4) The rate of loss of fertilizing power is roughly proportional to the dosage of radiation.

(5) The motility of sperm is impaired and cleavage is delayed and abnormal. Development is differentially modified. (See previous paper, Hinrichs, '26*b*.)

(6) Ultraviolet radiation produces sperm agglutination.

(7) Fertilizing power decreases more rapidly than does motility in both radiated and nonradiated suspensions.

(8) Fertilization is incomplete when normal eggs are fertilized by radiated sperm, and may in some cases lead only to membrane formation.

(9) The loss of fertilizing power is probably due to the loss from the sperm, by outward diffusion, of some substance necessary for fertilization. Ultraviolet radiation augments the loss of fertilizing power, presumably by altering the surface of the

sperm, and thereby increasing its permeability to such a substance.

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THE SEASONAL INFESTATION OF *NASSA OBSOLETA* (SAY) WITH LARVAL TREMATODES.

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The purpose of this investigation has been to make a twelve months' survey of large numbers of a single species of marine mollusk, to determine what different larval trematodes parasitize it, and especially to determine and to try to interpret the seasonal fluctuations in the degree and character of the infestation. The study was begun at the Marine Biological Laboratory, Woods Hole, Massachusetts, in August, 1924, when several common species of gastropod were collected in Quamquisset Harbor. Examination at that time showed *Nassa obsoleta*, the common mud snail, to be the most heavily parasitized, and therefore this species was chosen as the one for study throughout the year. The biology and ecology of this snail have been studied by a number of investigators (see especially Dimon, 1905; Sumner, Osburn, and Cole, 1913; Allee, 1923*a*, 1923*b*). The work was continued at the Zoölogical Laboratory of Washington University with snails shipped periodically from Woods Hole, and the twelve months' survey was completed at Woods Hole during the summer of 1925. All collections were made from a fifty yard area in the part of Quamquisset Harbor known as Gansett; a total of 8,875 individuals of *Nassa obsoleta* were examined. In addition to the data on the seasonal infestation brief descriptions of the larval trematodes are also included.

HISTORICAL.

Relatively little work has been done on marine larval trematodes, and that chiefly by European investigators. Among these Pelseneer (1906), Sinitsin (1911), and Lebour (1905–1912) have

[1] The routine examination of snails and collection of data for descriptions of the trematodes are almost wholly the work of the junior author, as are all drawings except numbers 2, 9, 11 and 17.

contributed most extensively. There are only a few scattered references on North American cercariæ. Fewkes (1882) briefly described a cercaria with caudal setæ found free near Newport, Rhode Island. Tennent (1906, 1909), worked out the life history of *Bucephalus haimeanus* and described its gasterostome cercaria. Linton (1915*a*, 1915*b*) found three species of cercariæ in the Woods Hole region: two furcocercous forms, one from *Hydroides dianthus* and one from *Pecten irradians*, and one tailless larva from *Nassa obsoleta* which has been designated *Cercariæum lintoni* in this paper. More recently one of us (Miller, 1925*a*, 1925*b*) has made surveys of the larval trematodes infesting marine gastropods from Puget Sound and from the Dry Tortugas.

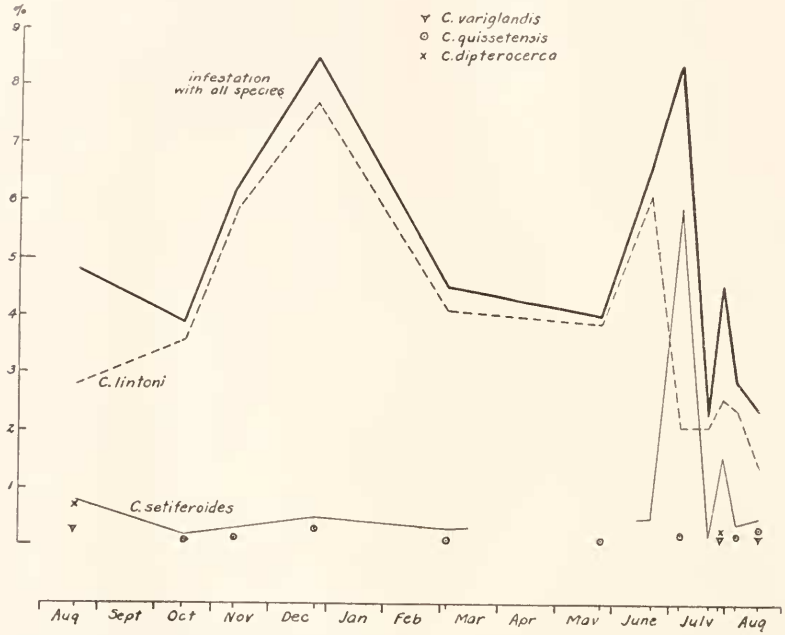
Material and Methods.

Snails received at Saint Louis were maintained until used in synthetic sea water made up according to the formula of Ditmar. All individuals of a collection, except that of May, were isolated for forty-eight hours to determine those from which mature larvæ were emerging. These infested snails were placed in separate aquaria as a constant source of living cercariæ for study. At convenient times the remaining snails were crushed individually, and their tissues examined under a binocular dissecting microscope in order to find non-emerging cercariæ and their parthenitæ; these were usually present in the digestive gland. After the living material had been studied, the infested viscera, freed cercariæ, and parthenitæ were fixed in Bouin's, or hot corrosive sublimate fluid. Cercariæ and parthenitæ were stained with Ehrlich's acid hematoxylin and mounted in Canada balsam. Unless otherwise noted, all measurements recorded were taken from these preserved specimens. Sections of snail viscera, two and one half and five micra in thickness, were variously stained.

Seasonal Infestation.

The graph for total infestation (Text-fig. 1) with all five species of larvæ has two maxima, practically equal, occurring in December and in July. Between these maxima are low areas, in each of which the percentage of infestation averages not more than one half that of the maximum. This plainly shows a seasonal fluctuation in the presence of larval trematodes in this

particular host. Before discussing this, the frequence and nature of the infestation with each of the five larvæ found will be taken up. Two of them were present only infrequently and in low percentages, and may be dismissed with brief statements. *Cercaria dipterocerca* sp. nov. was found in only four specimens, three in August, 1924, and one in late July, 1925; and similarly, *C. variglandis* sp. nov. was found in one host in each of these



TEXT-FIGURE 1. Graphs showing seasonal fluctuation in total infestation of *Nassa obsoleta*, and infestation with each species of trematode.

same collections, and once in August, 1925. There is no obvious explanation for the slight and infrequent occurrence of these two trematodes in *Nassa obsoleta*. It may be that they are normally parasitic in some other mollusk host, but are capable of developing occasionally in this species of *Nassa;* or the final hosts may be migratory.

A third species, *C. quissetensis* sp. nov., was found in eight of the twelve collections, always in very few hosts, and never emerging after isolation of the snail. In the October collection the rediæ were filled with mature cercariæ; in November and

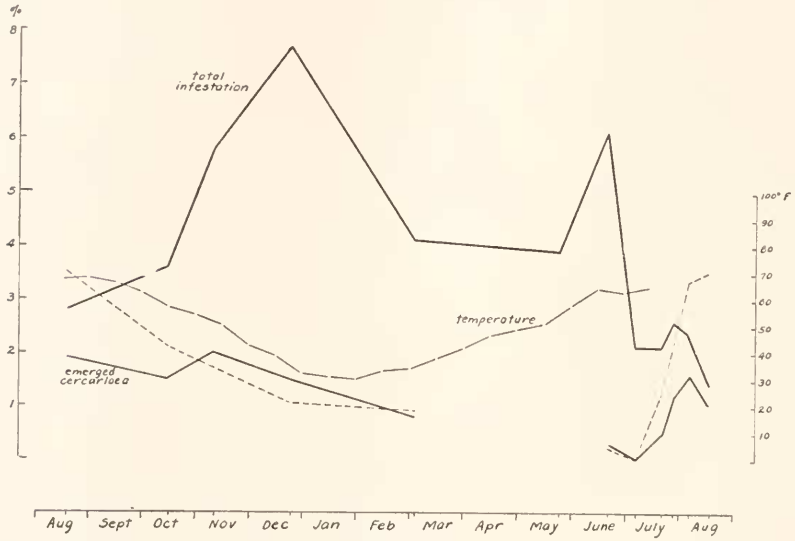
December only immature rediæ were present, while in the succeeding months there were always some apparently mature, but non-emerging cercariæ. In July and August, 1925, many fully developed cercariæ together with a few early germ balls were present in all of the rediæ. A fourth species, *C. setiferoides* sp. nov. was found in every collection but that of May. Although usually less than one per cent. of *Nassa* was infested, in early July (1925) 5.9 out of a total of 8.4 per cent. infestation was due to this species. Some mature, or at least apparently fully formed, cercariæ were found in every collection, which would seem to indicate that *Nassa obsoleta* is continually being infested by the miracidia of this trematode. There was no emergence of *C. setiferoides* from isolated snails until June and July, 1925; this seems to be the season of maturity of this larva, although some infestations with immature rediæ were found both in late July and in August, 1925.

Much the greatest part of the total infestation was due to the presence of a tailless larva, *Cercariæum lintoni* sp. nov., which Linton (1915*b*) briefly described but did not name. It was present in every collection, and except in that of July 6, 1925, the percentage of infestation with this species was greater than the total for all other trematodes present (Text-fig. 1). Although fully developed larvæ were found throughout the year, there was a striking seasonal variation in the percentage of snails infested with mature [1] larvæ. In Text-figure 2, the total percentage of infestation of *Nassa obsoleta* with *Cercariæum lintoni* and the percentage of snails from which mature larvæ emerged are shown graphically. The two maxima of the total infestation graph might be ascribed to the semi-annual visitation of Woods Hole by the definitive vertebrate hosts, whether migratory birds or fishes. It is presumed that the majority of the definitive hosts of marine cercariæ are fishes, concerning the migrations of which

[1] The emergence of fully formed larvæ from the snail when isolated in sea water for forty-eight hours is taken as evidence of the full maturity of the trematode. In some cases the larvæ in crushed snails were apparently fully formed, but did not emerge, for reasons unknown. Whether or not emergence is a fair test of maturity, the same procedure has been followed with all collections (except the May shipment, when they were not isolated due to lack of time). It is possible that in the case of some snails only a few larvæ emerged and that these escaped observation.

relatively little is known (Meek, 1916; Bigelow and Welsh, 1925).

Why a certain percentage of infestation, such as the maximum of December or June, once reached is not maintained would seem to be due directly to two factors, the death of heavily infested snails, and the recovery of some of the snails because of the maturing and complete emergence of the larval trematodes. The serious and often fatal effects of larval trematode parasites on the snail host have been studied by a number of investigators.



TEXT-FIGURE 2. *Cercariæum lintoni;* graphs showing total infestation and percentage of emerged (mature) cercariæ.

The very rapid increase in actively metabolizing trematode tissue, which frequently results in the destruction of most of the visceral mass of the snail, probably causes the death of large numbers of them. As to recovery, Sewell (1922: 17) and others have noted a condition of degeneration of liver or gonad apparently attributable to previous trematode infestation; the present author has also found this condition in some individuals of each of a number of species, such that at first glance the liver appeared to be parasitized, but no trematodes were present. Sewell included also the factor of natural death at certain seasons of the year as one which might explain seasonal fluctuations in percentage of

infestation. This operates, according to him, to raise the percentage of infestation by removing old snails which had formerly been infested and had recovered and become immune. He believes that the majority of fresh water mollusks may die from natural causes in June and July. This is borne out by the observations of Manson-Bahr and Fairley, Annandale, and others. This factor is probably an important one among the fresh water snails of India which Sewell studied, for the percentage of infestation was frequently very high and the number of old, immune individuals was probably relatively quite large. But among marine gastropods, where usually only a small percentage harbors larval trematodes, it would not be expected that this factor

TABLE I.

INFESTATION RECORD OF 8,875 SPECIMENS OF *Nassa obsoleta*.

Date.	Number Collected.	Died before Examination.	Total Per Cent. of Infestation.	Infestations.	Per Cent. of Infestation.
8/17/24	353	0	4.8	10, *C. lintoni* (7, emerged).	2.8
				3, *C. setiferoides* (2, immature rediæ only).	0.8
				3, *C. dipterocerca* (1, emerged).	0.8
				1, *C. variglandis*.	0.3
10/14/24	869	2	3.9	31, *C. lintoni* (13, emerged).	3.6
				2, *C. setiferoides* (1, immature rediæ only).	0.2
				1, *C. quissetensis*.	0.1
11/11/24	995	3	6.2	58, *C. lintoni* (20, emerged).	5.8
				3, *C. setiferoides* (2, immature rediæ only).	0.3
				1, *C. quissetensis* (immature rediæ only).	0.1
12/24/24	947	9	8.5	73, *C. lintoni* (15, emerged).	7.7
				5, *C. setiferoides* (2, immature rediæ only).	0.5
				3, *C. quissetensis* (immature rediæ only).	0.3
3/3/25	1,153	9	4.5	47, *C. lintoni* (9, emerged).	4.1
				4, *C. setiferoides*.	0.3
				1, *C. quissetensis*.	0.1
5/22/25	727[1]	8	4.1	29, *C. lintoni*.	4.0
				1, *C. quissetensis*.	0.1

[1] Not isolated for emergence.

TABLE I.—(*Continued*).

Date.	Number Collected.	Died before Examination.	Total Per Cent. of Infestation.	Infestations.	Per Cent. of Infestation.
6/20/25	649	1	6.6	40, *C. lintoni* (2, emerged). 3, *C. setiferoides* (1, emerged).	6.2 0.5
7/6/25	509	0	8.4	11, *C. lintoni* (0, emerged). 31, *C. setiferoides* (7, emerged). 1, *C. quissetensis*.	2.1 5.9 0.2
7/21/25	575	0	2.3	12, *C. lintoni* (3, emerged). 1, *C. setiferoides*.	2.1 0.2
7/29/25	504	0	4.6	13, *C. lintoni* (6, emerged). 8, *C. setiferoides* (3, emerged, 4, immature rediæ only). 1, *C. dipterocerca*. 1, *C. variglandis*.	2.5 1.6 0.2 0.2
8/ 6/25	546	0	2.9	13, *C. lintoni* (9, emerged).	2.4
8/15/25	423 [2]	0	0.7	3, *C. lintoni* (2, emerged).	0.7
8/18/25	625	0	2.4	9, *C. lintoni* (6, emerged). 3, *C. setiferoides* (very immature). 2, *C. quissetensis*. 1, *C. variglandis*.	1.4 0.5 0.3 0.2
Total...	8,875				

would play a considerable part. There are no data in Dimon's (1905) study of the biology of *Nassa obsoleta* on the life span of this species, nor whether large numbers die at any particular season of the year. The question of age immunity does not enter into the present study, as only data for large specimens of *Nassa* were included in the graphs; one collection of small individuals showed an infestation of only 0.7 per cent. on August 15, 1925, in contrast to 2.4 per cent. (Aug. 6) and 1.4 per cent. (Aug. 18) for two collections of large ones.

Inspection of the graph for emerged cercariæa (Text-fig. 2) shows that in the different collections there is variation in the percentage of snails harboring mature larvæ. There does not seem to be any correlation between these percentages and the

[2] Small snails; data not included in any graph.

fluctuations in the total percentage of infestation; the relative percentage of mature larvæ to total infestation is shown by the dotted line. It is unfortunate that the May collection was not tested for emergence; but from the available data it is seen that the occurrence of mature larvæ continues high from August to December, and then apparently is lower until late July and August, at which time it approximates its former values. The high percentages of emerged *C. lintoni* in July and August, 1925, are supported by the fact that during these months most of the sporocysts from crushed snails contained fully formed larvæ. Plotting of the temperature readings taken at the Fish Commission Wharf,[1] probably only in a general way comparable to that at Quamquisset Harbor, seems to show a relation between maturity and temperature; the maturity graph apparently lags behind that of temperature, remaining high while the temperature is dropping in November and December, and low for a considerable period after the temperature has increased in the spring and early summer months. Other factors which have already been discussed as affecting total infestation might also affect the percentage of mature cercariæa.

It is interesting to note that there is a close general similarity between the graphs shown by Sewell (1922: Chart 1) for two species of fresh water snails from India and the total infestation graph for *Nassa obsoleta* in the present study. The maxima for Sewell's examination of *Melanoides tuberculatus* from the Museum tank, fall in July and December, as they do for *Nassa obsoleta*, and the partial graph for *M. tuberculatus* from the Zoölogical Gardens follows the same general trend, with summer maximum in August rather than in July. Sewell's two graphs are based on 139 and 53 mollusk individuals respectively, while in the present study 8,452 large, and 423 small, specimens of *Nassa obsoleta* were examined.

[1] Temperature data were secured from the records of the Bureau of Fisheries for the 1st and 15th of each month of the period from August, 1924, to August, 1925; the figures are the mean of three daily readings taken at the U. S. Fisheries Station at 8 A.M., 12 M., and 4 P.M. The highest is 71° F. for August 1, 1924, and the lowest 30° F., just six months later, February 1, 1925. Thus there was at least an annual range of 41° Fahrenheit.

Summary.

From the data resulting from the examination of almost nine thousand specimens of *Nassa obsoleta* Say it seems clear that there is a semi-annual rise and fall in the larval trematode infestation. In view of the fact that none of the adults of these larvæ are known it is difficult to explain these phenomena. In all probability migrations of the definitive hosts, and the degree of their infestation, affect the seasonal fluctuations; and other factors are probably the life span of *Nassa*, and the effect of parasitism upon it. The relative importance of these factors is not clear.

Brief Description of New Species Cited in this Paper.

Cercaria setiferoides sp. nov.

(Figs. 1–3, 6, 10.)

Trichocercous distome cercaria with opaque yellowish body and characteristic excretory vesicle. Contractile body varying from 140 μ to 268 μ in length and 104 μ to 156 μ in width; average length 187 μ and width 126 μ; average tail length 486 μ. Oral sucker slightly elongate, 64 μ in average length and 34 μ in width; ventral sucker smaller and oval, 40 μ in width and 34 μ in length. Surface of body finely pebbled, and completely covered with short, regularly arranged spines. Conspicuous pigmented eye spots, composed of large spherical granules and a so-called lens. No spines on tail, but on either side of it rows of long setæ; usually seven setæ per group, the longest in the center; about thirty pairs of setæ groups arranged along sides of tail opposite to each other. Well developed digestive system clearly seen in both living and preserved material; mouth antero-ventral in oral sucker; short prepharynx followed by large oval pharynx, 24 μ in length and 16 μ in width; short esophagus, and two wide intestinal ceca extending to posterior end of body; jelly-like contents of ceca with great affinity for intra-vitam neutral red, in contrast to all other structures in body. Eleven pairs of larval glands, preacetabular in position, arranged in three groups; no observable ducts from the most posterior six glands; glands of all groups strongly acidophilic in all combinations of stains;

lightly stained with intra-vitam neutral red, but not with toluidine blue. Large excretory vesicle, the most conspicuous of internal structures, slightly to right of median line normally, and extending nearly to region of bifurcation of gut; very wide and bent more or less in shape of Z; filled with spherical refractile concretions, ranging from very small ones up to 3 μ in diameter. Main lateral collecting tube of either side entering excretory vesicle at a point about two thirds of distance from anterior end; entrance of anterior and posterior branches posterior to level of ventral sucker. Succession of single excretory flagella in wall of main lateral collecting tube; many flame cells observed, but exact pattern of excretory system not solved; apparently more highly developed posteriorly. Several cell masses present in anlage of reproductive system; no interpretation ventured as to parts of adult system represented. Development of cercariæ in rediæ in visceral mass of *Nassa obsoleta*. Immature rediæ slender, without locomotor appendages; average length 440 μ, and diameter at widest part 74 μ; long coiled gut extending beyond middle of redia. Germinal epithelium localized in posterior end. More mature rediæ with both mature cercariæ and germ balls.

C. setiferoides is similar to *C. setifera* Joh. Müller 1850 (described by Monticelli, 1914, and redescribed by Odhner, 1914, from one of Monticelli's slides), but differs in a number of important structures. It is obviously different from *C. lutea* Giard 1897, *C. pectinata* Huet 1891, and *C. setifera* Pelseneer, 1906; *C. fascicularis* Villot 1875 is not completely described. The cercaria with caudal setæ which Fewkes (1882) found free near Newport, R. I., may be identical with *C. setiferoides;* a detailed description of it was not published. The present species may also be identical with the larval form of *Pharyngora bacillaris*, recorded by Nicoll (1910) and Lebour (1917) as free in the plankton from Plymouth. The latter investigator (1916, 1917) found the metacercarial stage in various medusæ and in *Sagitta bipunctata;* her brief description and single figure of the trichocercous cercaria (1917) are not sufficiently detailed to determine whether it and the present species are the same.

Cercaria dipterocerca sp. nov.

(Figs. 4, 5, 7, 8.)

Distome larva with prominent lateral cuticular fins extending along entire length of tail. Average body length 145 μ, varying between 109 μ and 212 μ, width 50 μ, varying between 47 μ and 65 μ. Body, but not tail, uniformly covered with small spines; double row of large ones around mouth. Pigment present only in two large eye spots, composed of very coarse granules and a "lens." Oral sucker 24 μ in diameter, larger than ventral sucker; subterminal mouth, prepharynx, pear-shaped pharynx; ceca not seen posterior to ventral sucker. Nine pairs of three different kinds of larval glands: on either side two pairs (Fig. 7, *a*), dorsal, lateral to pharynx, with coarsely granular, yellowish cytoplasm; stain with intra-vitam neutral red; eosinophilic in sections; second group of four pairs (*b*), laterally situated, with finely granular, greyish cytoplasm; basophilic with Ehrlich's acid hematoxylin; third group of three pairs (*c*), not observed in living cercaria; slightly basophilic in sections; no ducts found. Large thick walled excretory vesicle, varying from elongate oval to triangular in outline; main lateral collecting tubes and positions of some flame cells shown. Reproductive system represented by conspicuous mass of cells dorsal to ventral sucker. Tail with a pair of lateral convoluted cuticular fins extending the entire length; smaller median fin on ventral side of distal fourth of tail, extending around end up on to dorsal side; maximum tail length 320 μ, average 250 μ. Development of cercaria within elongate rediæ, averaging 795 μ in length and 92 μ in diameter; pharynx large; more or less cylindrical redia constricted at irregular intervals.

Cercaria dipterocerca differs from *C. hymenocerca* Villot 1875, *C. quadripterygia* Sinitsin 1911, and *C. lophocerca* (in Lebour, 1912) in significant details of structure.

Cercaria variglandis spec. nov.

(Figs. 15–17.)

Binoculate furcocercous distome cercaria most closely resembling members of the Elvæ group (Miller, 1923:44). Average

body length 262 μ and width 77 μ; tail approximately same length as body; ratio of tail stem to furcæ about 3 : 2. Anterior penetrating organ, a highly modified oral sucker, 51 μ in greatest diameter; ventral sucker much smaller. Eosinophilic head gland observed in sections of anterior organ; ventral capillary mouth and esophagus as in other members of group. Body covered with short spines uniformly distributed; absent from tail and furcæ. Two eye spots composed of large pigment granules, in posterior connection with nervous system. Most of body filled with three differentiated sets of larval penetration glands: one pair of cells, posterior to eye spots, with finely granular cytoplasm, chromophobic in sections; two pairs of glands, dorsal to ventral sucker, with coarsely granular eosinophilic cytoplasm; three pairs of glands in posterior part of body, with rod-filled cytoplasm, homogeneous in sections, staining deeply with iron hematoxylin. No rapid selection of intravitam neutral red or toluidine blue shown by any set; anterior pair deeply stained, the two middle pairs chromophobic, and the three posterior pairs lightly stained in very strong solution of either dye. Five pairs of flame cells in body and one pair in proximal tail stem; exact connections of two posterior body flame cells not determined, but judging from fresh water larvæ for which excretory system is known pattern is probably like that of *C. wardi* (Miller, 1923: Text-fig. 4); excretory system in tail opening at tips of furcæ. Cell mass representing future reproductive system ventral and posterior to ventral sucker. Development of cercaria within long sporocysts of uniform diameter throughout, measuring on average 1.3 mm. in length and 0.37 mm. in width and containing between twenty and thirty cercariæ.

Cercaria variglandis is different in a number of respects from the few described marine furcocercous larvæ: *C. dichotoma* Müller 1850, *C. discursata* Sinitsin 1911, and *C. syndosmyæ* Pelseneer 1906.

Cercaria quissetensis sp. nov.

(Figs. 9, 11–14, 18, 19.)

Echinostome cercaria with twenty-seven spines on collar. Body averaging 290 μ in length and 130 μ in width (extreme

extension of living cercaria 630 μ); tail about 330 μ in length. Oral sucker 42 μ, ventral sucker 64 μ in diameter. Mouth subterminal, short prepharynx, pharynx, wide esophagus bifurcating into ceca extending to extreme posterior part of body. Granular cytoplasm of small glands around esophagus and portions of contents of esophagus and ceca stained deeply with neutral red; dorsal gland cells eosinophilic and ventral ones chromophobic in sections; fine bundles of minute ducts passing through oral sucker and opening on anterior end. Many rod-filled cystogenous glands [1] in dorsal part of body; stained deeply with iron hematoxylin in sections. Excretory vesicle averaging 36 μ in diameter; arms filled with refractile concretions, of double coffee bean shape. Excretory system pattern not completely worked out; of type of *C. complexa* Faust (1919); twelve to fourteen flame cells observed on one side. Irregular masses of reproductive system cells posteriorly located, with a row of nuclei extending to a small mass anterior to ventral sucker. Average length of sausage-shaped redia 1.1 mm., width 0.2 mm.; orange-yellow pigment in wall; birth pore and two posterior locomotor appendages observed only in immature rediæ. Cercariæ encyst readily on glass slide, cysts averaging 142 μ in diameter, with two diametrically opposite projections.

Cercaria quissetensis differs in a number of respects from the six species reviewed by Lebour (1912), and from *C. proxima* and *C. sagitata* Lespès.

Cercarieum lintoni sp. nov.

(Figs. 20, 21.)

Tailless larva, properly designated Cercariæum; original description by Linton (1915*b*), supplemented and emended in a few details by this study. Average length of a number of emerged larvæ, killed without pressure, 230 μ, width 84 μ. Very

[1] There is a possibility that the October and November infestations represent a second species of echinostome. The second infestation consisted of immature rediæ only, but in October the rediæ were filled with apparently fully formed cercariæ (Fig. 12). These differ from the mature cercariæ of the succeeding infestations chiefly in that they are of somewhat smaller size and different shape (Fig. 13), and lack cystogenous material. This is not considered as sufficient to differentiate them into two species.

narrow intestinal ceca, traceable only in serial sections, reaching almost to excretory vesicle. One pair of prominent larval gland ducts, with granular contents, on either side of body, mistaken by Linton for excretory vesicles; four large eosinophilic larval glands, staining also with intra-vitam neutral red, but clearly observable only in sections. Large excretory vesicle, much constricted posteriorly, and surrounded by a sphincter muscle; opening in center of adhesive disc. A few flame cells observed, but exact pattern of excretory system obscured by numerous refractile globules distributed throughout body. Future reproductive system represented by a number of cell groups; two spherical masses, just posterior to ventral sucker, probably testes, with female complex anterior to excretory vesicle. Inchworm locomotion of this species effected by successive attachment of posterior end of body, modified by invagination into an adhesive disc, then extension of body and attachment by ventral sucker.

Cercariæum lintoni is obviously different from *C. dentali*, *C. giardi*, and *C. crispata* of Pelseneer (1906), and from the five species described by Lebour (1912) in character of the digestive and excretory systems, or in the parthenita.

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Plate I.

All drawings were made with the aid of a camera lucida, except where otherwise stated.

Fig. 1. *Cercaria setiferoides;* general view. × 150.

Fig. 2. Free-hand diagram of excretory system pattern in redia of *C. setiferoides.* × 40.

Fig. 3. Immature redia of *C. setiferoides*, showing pharynx and gut. × 92.

Fig. 4. *C. dipterocerca;* immature redia. × 88.

Fig. 5. General view of *C. dipterocerca.* × 56.

Fig. 6. Nearly mature redia of *C. setiferoides*, containing germ balls and almost fully formed cercariæ. × 72.

Fig. 7. *C. dipterocerca;* dorsal view of body showing eye spots, digestive and gland systems, and locations of most easily observable flame cells. Reproductive system cell mass between ventral sucker and excretory vesicle. × 415.

Fig. 8. Nearly mature redia of *C. dipterocerca.* × 138.

Fig. 9. Outline of body of *C. quissetensis*, when in extreme extension. × 42.

Fig. 10. *C. setiferoides;* dorsal view of body showing eye spots, digestive and gland systems, and cell masses representing future reproductive system. Only excretory vesicle and entrance of main lateral collecting vessels are shown. × 342.

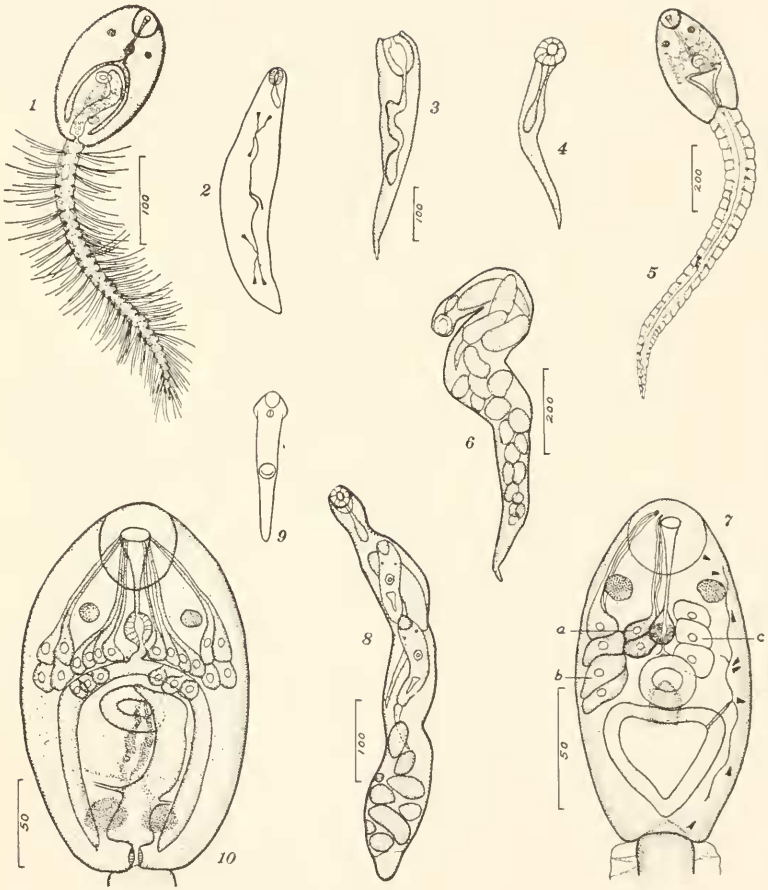

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Plate II.

Fig. 11. *C. quissetensis;* redia with gut and birthpore, containing only one fully formed cercaria. × 53.

Fig. 12. Redia of *C. quissetensis*, packed with mature cercariæ. × 47.

Fig. 13. Ventral view of body of *C. quissetensis;* digestive and gland systems; main trunks of excretory system; cell masses of future reproductive system. × 162.

Fig. 14. General view of *C. quissetensis.* × 93.

Fig. 15. *C. variglandis;* dorsal view showing anterior organ (highly modified oral sucker), eye spots, and three differentiated sets of gland cells. × 120.

Fig. 16. Sagittal section of body of *C. variglandis*, showing especially the different gland cells. × 385.

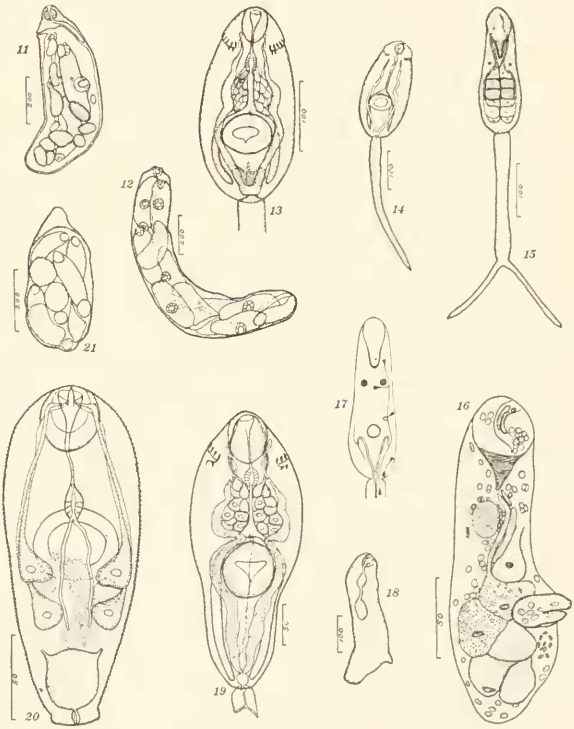
Fig. 17. *C. variglandis;* free-hand diagram of flame cell pattern in body. × 190.

Fig. 18. *C. quissetensis;* immature redia, showing pharynx and gut, and posterior locomotor appendages (on one side only). × 83.

Fig. 19. Extended body of *C. quissetensis*, showing digestive, gland and excretory systems, and reproductive system cell masses. × 200.

Fig. 20. *Cercariæum lintoni;* dorsal view showing digestive and gland systems, cell masses of reproductive system, and excretory vesicle. × 398.

Fig. 21. Sporocyst of *C. lintoni*, containing germ balls and mature cercariæ. × 70.



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CORRELATIONS AND VARIABILITY OF THE CENTRAL NERVOUS SYSTEM AND BODY SIZE OF THE ALBINO RAT.

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A knowledge of inter-organ weight relationships gives data which assist in the making possible a more exact conception of differential development. The presence or absence of a statistical association may indicate the presence or absence of a community of growth response to general developmental factors; it may indicate the presence or absence of a specific conditioning of the one organ by the other; or it may indicate the play of reciprocal influence.

The use of the coefficient of correlation in an investigation of this problem is marked by its neglect. What little the literature does contain, is, with one or two exceptions, sketchy and inadequate. It is usually a mere record of figures which in many cases are of meagre value because of the paucity of raw data.

Realizing the need for a more systematic survey of inter-organ weight relations a beginning was made somewhat over a year ago with a biometrical analysis of the weight interrelations of the glands of internal secretion. A preliminary note was published in Endocrinology (1) in anticipation of full presentation in the *Journal of Metabolic Research* at some future date. In the latter there will be found the conditions which should govern a study of this nature. The present paper is an extension of the analysis to the brain and spinal cord, using as raw data the body weights, body lengths, brain weights and spinal cord weights of the same rats the organ weights of which served for the earlier study. These animals were the controls of the studies of the thyroid apparatus (2). They were 150 days of age at the time the organs were removed for weighing. Both sexes were used, 121 rats of each. They all came from the Experimental Colony Stock of The Wistar Institute. The values thus represent the associations found in the mature male and female albino rat of this stock.

Turning now to the method of analysis I can do no better than to quote directly from Miner (3): "A coefficient of correlation measures the degree to which two variables are associated, taking the value + 1 when a deviation of one variable from its mean is always associated with a proportional deviation of the other in the same direction, decreasing as the intensity of the association decreases until for complete independence of the two variables it takes the value 0, and again increasing in numerical value, but with a negative sign for increasing intensity of association where deviations of one variable in one direction are coupled with deviations of the other variable in the opposite direction. Thus the absolute magnitude of a coefficient of correlation measures the intensity of the association of two variables, while its sign indicates whether as one variable changes the average values of the other variable change in the same or opposite directions. The possible range of values is from + 1 to − 1."

The coefficient of correlation does not of itself indicate which is the dependent and which the independent variable. This must be determined by other means. The simple or zero order correlation between any pair of variables is not representative of their association uncomplicated by assumed interferences derived from the other variables. In order to obtain such an uncomplicated index it is necessary to determine the correlation between any given pair of variables when the others are held constant. This is done by the method of partial correlation.

Now it is conceivable that the weight association between brain and spinal cord is factored by the general size factors carried by the body weight and possibly by the body length, particularly in the case of the spinal cord. Hence the elimination of these general factors for size by stabilization for body weight and length, is essential if we are to obtain the uncomplicated association between the brain and the spinal cord. That is to say the association free from the general body size factors. This has been done as succeeding paragraphs will show.

In order that the values obtained by the method of partial correlation be valid it is necessary that all the zero order regressions be linear, and that the number of observations in each of the zero order tables be fairly large as compared with the

number of variables dealt with. The test for linearity is made through a determination of the correlation ratio and a comparison of this with the coefficient of correlation r. The methods used for the determination of the coefficient of correlation, the correlation ratio and the partial correlation coefficient are those described by Pearl (4).

The raw data and the correlation tables which serve as the basis of this study are not given because of space limitations. They are on file at The Wistar Institute.

In Table I. are given the zero order coefficients of correlation (r_0), the correlation ratios (η), the values for ($\eta^2 - r^2$), the probable errors for r_0 and ζ, and the quotients r/E_r and ζ/E_ζ of the several comparisons made in this study. In addition to these values there is to be found in the literature the following figures: Donaldson (5), (6) in a series of rats of scattered ages and with the sexes combined found the correlation between body weight and brain weight to be 0.764: the correlation between body weight and cord weight to be 0.856: between body length and brain weight to be 0.86: between body length and cord weight to be 0.99. Hatai (7) records a value of 0.516 in the male and of 0.692 in the female for the coefficient of correlation between body weight and cranial capacity in the mature albino rat. Jackson's (8) values are based on too few observations to be any more than suggestive. For adult man, Boas (9) records a correlation between body weight and head length of 0.43 in the male and 0.41 in the female; and between body weight and head width of 0.32 in the male and 0.33 in the female. Blakeman (10) found the coefficient of correlation between body length and brain weight to be 0.289 in the male and 0.367 in the female; while Pearl (11) got values ranging from 0.166 to 0.183 in the male and from 0.183 to 0.349 in the female for this association, and 0.167 in the male and 0.226 in the female for the degree of association between body weight and brain weight.

The values for man are of little help in the problem because the raw data from which they were derived are complicated by a multiplicity of interfering variables which vitiate any comparison with the figures obtained from the more uniform population of albino rats.

Table I.

The Coefficients of Correlation, the Correlation Ratios and the Quotients of the Errors of the Several Comparisons.

Sub-script.	Male.					Female.				
	r_0.	r/E_r.	η.	ζ.	$\zeta/E\zeta$.	r_0.	r/E_r.	η.	ζ.	$\zeta/E\zeta$.
12	0.872 ± 0.015	58.1	0.882	0.0175 ± 0.0161	1.1	0.783 ± 0.024	32.6	0.811	0.0446 ± 0.0255	1.8
13	0.676 ± 0.033	20.5	0.725	0.0686 ± 0.0310	2.2	0.710 ± 0.030	23.7	0.755	0.0659 ± 0.0305	2.2
14	0.809 ± 0.021	38.5	0.821	0.0195 ± 0.0170	1.2	0.726 ± 0.029	25.0	0.770	0.0658 ± 0.0305	2.2
23	0.650 ± 0.035	18.6	0.701	0.0689 ± 0.0310	2.2	0.571 ± 0.041	13.9	0.632	0.0734 ± 0.0316	2.3
24	0.778 ± 0.024	32.4	0.798	0.0315 ± 0.0215	1.5	0.719 ± 0.030	24.0	0.751	0.0470 ± 0.0260	1.8
34	0.800 ± 0.022	36.4	0.813	0.0210 ± 0.0176	1.2	0.779 ± 0.024	32.5	0.799	0.0316 ± 0.0215	1.5

(1) Body Weight; (2) Body Length; (3) Brain Weight; (4) Spinal Cord Weight.

Notwithstanding the non-elimination of the age factor, the associations reported by Donaldson (6) between body weight and brain weight, body weight and spinal cord weight, and the brain and spinal cord in his series of rats are of the same order of magnitude as those found in this study. The association between cranial capacity and body weight in the female as recorded by Hatai (7) is also of the same order of magnitude as my value for body weight and brain weight correlation. Aside from these similarities the data are rather widely divergent.

Turning now to the analysis of my data it is evident that there is a high degree of positive correlation between the several pairs of variables under investigation. Neglecting the brain-spinal cord weight correlation ($r_{3\ 4}$) it is seen that the order of increasing degree of association is the same in both sexes. This indicates that the association of the brain and spinal cord with the body as a whole is governed by factors which are largely independent of sex determinants of association. These independent factors are probably specific in origin.

The degree of association between brain weight and spinal cord weight ($r_{3\ 4}$) is practically the same in the female as in the male. This indicates that the correlation is independent of the sex differences in body size which exist in animals of the same age. That is to say the association between these two parts of the central nervous system is independent of sex factors contributive to differences in differential development. It is, however, dependent on other factors. While it is possible that specificity plays an important part in the determination of the association, I am inclined to believe that the basis of the reaction lies rather in the community of characteristic chemical make-up of the two organs, with the consequent similarity in response or resistance to extraneous influences. Not to be neglected is the idea that the chemical similarity conditions a similarity in the processes of growth and hence association in weight.

The association between body weight and body length ($r_{1\ 2}$) is consistently greater than that between the other pairs of variables. Nevertheless, the superiority is statistically valid in but 50 per cent. of the comparisons. The general trend of difference is uniform, however, and if accepted as significant is

suggestive of a greater interdependence or dependence on a common factor. Body weight and body length are thus more closely related in differential development, than is the brain or spinal cord related to either of them individually. That is to say, body weight and body length follow more nearly parallel courses during differential development than do brain and body weight or body length, and spinal cord and body weight or body length. The difference can be attributed to the probability that the type metabolism productive of body weight is more nearly like that productive of body length, than is the type metabolism of body weight and body length like that which characterizes brain and spinal cord. In the case of body weight and body length the reactions are essentially increments in protoplasm, in the case of the brain and spinal cord the differential development is characterized by lipoid accretions.

The correlation between brain weight and body weight ($r_{1\,3}$) is less than that between the other pairs of variables with the exception of brain weight and body length ($r_{2\,3}$). The degree of difference is valid in 50 per cent. of the cases. The difference if accepted as significant is suggestive of a lesser dependence of brain weight and body weight on a common factor. That is to say brain weight and body weight are less related in differential development than are spinal cord weight and body weight and length, and than brain weight and spinal cord weight with each other. From which it can be inferred that during differential development brain weight deviates more from the course followed by body weight, than it does from that followed by the spinal cord, or than does the spinal cord deviate from the course followed by body weight and body length.

The greater association between brain and spinal cord ($r_{3\,4}$) is undoubtedly due to the community of type metabolism as compared with the disparity between brain and body weight. The higher degree of association between spinal cord and body length ($r_{2\,4}$) is justly attributed to the structural relations existing in this case. Why cord weight should be more closely associated with body weight ($r_{2\,4}$) than is brain weight is a question. It may be that the preference is a consequence of the structural relationship of the cord with the body length of high degree of correlation with body weight.

Both brain and spinal cord are more closely associated with body weight than with body length. Although the differences are slight and not statistically valid, the consistency of their direction and their presence in both sexes puts into the relation a significance that cannot be denied. It is a relation that might be expected by virtue of the fact that the developmental processes productive of weight in the parts of the organism are more closely allied than the developmental processes productive of weight are allied to those of length. This result would seem on the face of it to detract somewhat from Donaldson's (5) dictum that "body length is a better datum than body weight from which to infer the weight of the brain or spinal cord." The objection is negatived, however, by the fact that in a normal population the variability in body weight is greater than that in body length. This will be discussed presently.

The high degree of positive correlation of brain weight and spinal cord weight with body weight and body length allows the extension of Donaldson's (6) conclusion that the weight of the spinal cord can be inferred from body length or body weight with a high degree of accuracy, to include the brain.

With one exception ($r_{1\,3}$) the degree of association between the several pairs of variables is greater in the male than in the female rat. The degree of difference is, however, statistically valid only in the case of the body weight-body length correlation ($r_{1\,2}$). These figures in Table I., it must be remembered, are indices of the degree of association between pairs of variables, when interfering influences assumed to be exerted by the other variables are still present. Such being the case, and if the general trend of sex difference is accepted as significant because of its consistency, it is suggestive of a greater independence of the central nervous system of the female from the general factors contributive to interstructural and inter-organ association as carried by the body as a whole. This inference is supported by the results of the analysis by the method of partial correlations. The use of this method is allowable here because the regressions are in all cases essentially linear.

In Table II. are given the correlation coefficients of the several pairs of variables after the removal of the assumed influences

exerted by the others by statistical treatment. Continuing the phase of comparison dealing with the sex differences. If we take as a measure of this relation the sex difference in degree of change in association of the first and second order coefficients from the zero order correlation between brain weight and spinal cord weight ($r_{3\,4}$), *i.e.*, when first body weight ($r_{34\,1}$) or body length ($r_{34\,2}$) are held constant, and then when the body weight and body length are both held constant ($r_{34\,12}$), it is seen that in general the reduction in value is percentagely greater in the male than in the female. This indicates that the conclusion drawn in the preceding paragraph is justified.

Table II.

The (Partial) Correlation Coefficients.

Subscript.	Male.	Female.
r_{12}	0.872	0.783
r_{13}	0.676	0.710
r_{14}	0.809	0.726
r_{23}	0.650	0.571
r_{24}	0.778	0.719
r_{34}	0.800	0.779
$r_{12\,3}$	0.773	0.654
$r_{12\,4}$	0.659	0.546
$r_{13\,2}$	0.293	0.515
$r_{13\,4}$	0.082	0.334
$r_{14\,2}$	0.425	0.377
$r_{14\,3}$	0.606	0.391
$r_{23\,1}$	0.169	0.034
$r_{23\,4}$	0.074	0.025
$r_{24\,1}$	0.253	0.353
$r_{24\,3}$	0.566	0.532
$r_{34\,1}$	0.584	0.545
$r_{34\,2}$	0.616	0.644
$r_{12\,34}$	0.655	0.573
$r_{13\,24}$	0.043	0.384
$r_{14\,23}$	0.325	0.069
$r_{23\,14}$	0.027	0.201
$r_{24\,13}$	0.193	0.399
$r_{34\,12}$	0.567	0.570

(1) Body Weight; (2) Body Length; (3) Brain Weight; (4) Spinal Cord Weight.

Further confirmation of the conception is had from the fact that the growth of the brain and spinal cord of the female albino rat follows the changes in growth retardation in body weight and

body length which are caused by thyroid and parathyroid removal at different ages, to a lesser degree than does that of the male.

A general analysis of the progress of partial correlation would naturally only be an extension and confirmation of the comparisons made from the zero order values. As an example: the association between brain weight and spinal cord weight is conditioned to a lesser degree by body length than by body weight. This is shown by the fact that the percentage reduction in degree of association between brain and spinal cord weight from the zero order value ($r_{3\,4}$), is greater when body weight is held constant ($r_{34\,1}$) than when body length is held constant ($r_{34\,2}$). The difference is what is to be expected from the fact noted earlier that brain weight and spinal cord weight are more closely associated with body weight than with body length.

It is important to note that there is a high degree of positive correlation between brain weight and spinal cord weight ($r_{34\,12}$) which is independent of the general factors for size carried by the body as a whole. Indeed this value is much higher than that found for any of the pairs of organs so far studied (thyroid, adrenals, hypophysis, gonads, thymus and pancreas (1)). Between many of these no correlation was present at all after stabilization for body weight. Such being the case it is evident that the uncomplicated weight association between brain and spinal cord is peculiar. The phenomenon can be attributed to the community of characteristic chemical (lipoid) make-up of the two organs as already noted. As far as simple correlation with body weight is concerned, the brain and spinal cord have also a higher degree of association than any of the above, save the hypophysis and the pancreas in the male. The value of r_0 is 0.701 ± 0.031 for the hypophysis, and 0.600 ± 0.039 for the pancreas. It might be noted that since the same animals served as original sources of the material used in both studies, the value of the comparisons is enhanced.

There is no sex difference in the degree of association between brain weight and spinal cord weight freed from the general factors for size ($r_{34\,12}$). The significance of this relation has been discussed in an earlier paragraph.

Another statistical value of assistance in an estimation of the

forces concerned in differential development is the coefficient of variability when used comparatively. This figure is the quotient times 100 of the mean of the variates into their standard deviation, or, C.V. $= \sigma / M \times 100$ per cent. It is an abstract value which makes possible inter-group, inter-sex, inter-structural and inter-organ comparisons of sensitivity to the totale of forces contributive to variation which play upon the organism. In a study such as this, where a comparison is being made of the organs as parts of a whole, the differences in the coefficient of variability exhibited are indices of differences allied to differential development. They are worthy of investigation because they represent deep-seated biological relationships.

In Table III. are given the coefficients of variability and their probable errors of the body weight, body length, brain weight and spinal cord weight of the male and female albino rats at 150 days of age.

TABLE III.

COEFFICIENTS OF VARIABILITY OF THE BODY WEIGHT, BODY LENGTH, BRAIN WEIGHT AND SPINAL CORD WEIGHT OF THE MALE AND FEMALE ALBINO RATS 150 DAYS OF AGE.

Structure.	Male.	Female.
Body Weight	14.19 ± 0.63	11.39 ± 0.49
Body Length	4.42 ± 0.19	3.40 ± 0.15
Brain Weight	5.19 ± 0.22	4.92 ± 0.21
Spinal Cord Weight	6.69 ± 0.29	6.25 ± 0.27

The values show definitely that body weight in the female is less variable than in the male. This difference has already been noted by King for albino stock (12), for inbred albino stock (13) and for Norway rats (14). Jackson's (8) values show a like direction of difference in the albino rat.

This sex difference is not exhibited in adult man (11). Nevertheless it must be remembered that our data are derived from a racially homogeneous stock, while that of Pearl were not so constituted. It would be rash to state that the results of biometrical analysis of groups of humans is inadequate to divulge such relations. It is better to say that the lack of sex difference in value in a heterogeneous stock is no indication of its non-existence in an homogeneous population. While it would also be rash to generalize from rat to man, the fact that three observers

of five different rat populations have obtained sex-differences in body weight variability in the same direction, is indication that we are dealing with a biological sex-difference of response to factors contributive to variability, and that studies on man should be so planned as to eliminate the possible interfering factor of racial heterogeneity which might well mask a basic sex difference. Further evidence supporting this view is the fact that the body weight variability of the rat of homogeneous stock is generally considerably less than that of man of heterogeneous stock. The values recorded by Pearl (11) for man are 21.3 for the male and 24.7 for the female.

The female albino rat is also less variable than the male in body length to a statistically valid degree, and shows a like tendency in brain and spinal cord weight, though the degree of difference here is too small to be valid. At this time I do not want to go into a comparison of the entire array of organs in the rat, but might point out that the sex difference is not uniform in direction for all the organs, which fact has interesting implications as a later study will show.

However, the fact that in body weight and in body length the female is less variable than the male albino rat indicates in this species, at least, a greater stability of the female organism as a whole to outside forces tending to disturb body size equilibrium. Teleologically this might be considered an expression of a protective mechanism, tending to enhance resistance and thus favor the essential purpose of the female, namely reproduction.

From Table III. it is seen that body length is much more stable than body weight. The same holds true for man. The C.V. for stature in the male was found to be from 3.8 to 4.3 (Pearl) and 3.6 to 4.5 (Blakeman): and in the female from 4.0 to 4.7 (Pearl) and 3.8 to 4.2 (Blakeman). Attention is directed to the fact that the values for man are of the same order of magnitude as those for the rat.

It is hardly necessary to point out that the lesser variability of body length or stature is a consequence of the greater inherent metabolic stability of the skeleton as the chief component determinative of this measurement, as compared with that of the body weight with its predominant element of metabolically

variable tissue. The one is, by virtue of its chemical make-up, a relatively fixed structure, the other a fluctuating mass freely subject to many influences.

The fact that the body length variability of the rat is of the same order of magnitude as that of man is indicative of a biological similarity in inherent structural response to factors contributive to variability, which might well have been predicted from the very nature of the structures involved, and which is support for the idea expressed in a preceding paragraph that the species difference in body weight variability exhibited here is factored in part by difference in racial uniformity of population from which the data were derived.

The lesser variability of the body length combined with the fact that the variability in brain weight and spinal cord weight is closer in degree to that of body length than to that of body weight establishes the conclusion postulated by Donaldson (5) that body length is a better datum from which to infer brain and spinal cord weight, than is body weight.

Both brain and spinal cord are less variable than the body in weight. This also holds for man in the case of the brain. Pearl (11) records a C.V. value of 7.5 to 8.8 for the male brain, and 7.1 to 8.7 for the female, while Blakeman (10) found 7.8 for the male and 8.2 for the female.

The lower variability of the central nervous system is obviously again an expression of an inherently more stable chemical make-up. Evidence for this is had both in the fact that the brain and spinal cord are more resistant to conditions of malnutrition and inanition than is the body as a whole (15), and in the fact that the growth of these organs is more resistant to the metabolic upsets incident to thyroid and parathyroid deficiencies than is that of the body in weight (16). This has been discussed in another paper. All that need be pointed out here is that the high content of the central nervous system in characteristically stable lipoids determines in it a resistance to metabolic disturbances which primarily affect the more readily utilizable tissue components, such as occurs in inanition, thyroid deficiency and individual dietary idiosyncrasies affecting body weight.

As one after another point of view is used in the attack on the

problem of differential development, there emerges a unanimity of inter-relationship which substantiates the premises on which the interpretations are based.

The variability in brain weight is greater in man than in the rat. This is probably another expression of the difference between heterogeneous and homogeneous material. It is, therefore, not essentially a real difference. Contributive to, it may be the greater variability in human body weight as compared with rat body weight, and the high correlation between brain weight and body weight in the rat. The low association between this pair of variables reported for man rather negatives this idea, however.

Both brain and spinal cord are more variable than body length. In man also brain weight is more variable than stature. This may be taken as an indication that skeletal composition is more fixed and metabolically stable than is that of the central nervous system. Such a conclusion is in accord with the chemical and physiological data so far available.

The brain appears to be less variable than the spinal cord. This is consistent with the fact that brain weight is less highly correlated with body weight of high variability than is the spinal cord. On the other hand when body length variability is eliminated from brain and spinal cord variability free from the assumed influence of body weight variability, during the computation of the actual or reduced variability of these organs according to the usual statistical procedure, there is no reduction in value (of brain and spinal cord variability) below that which obtains when the variability has been stabilized for body weight. A different result would be expected if the respective brain and spinal cord associations with body weight and body length were dominant factors in the variability coefficients. Moreover, since the spinal cord has a relatively higher percentage of the stable (non-readily utilizable) lipoids characteristic of the central nervous system, and a relatively lower percentage of the labile readily utilizable elements than the brain, it would be expected that its (the spinal cord) variability would be less than that of the brain, if this compositional difference is the factor of importance. All the evidence so far accumulated gives an affirma-

tive answer to this last point (17), and hence the reversal of expected relationship is either a real objection to the theory or else some other factor has intruded to mask the normal reaction. Such a factor is present in the technic of removal of the spinal cord for weighing. This is no place to describe the matter, nor is it necessary, for a little consideration will show that when the removal of an organ is accompanied by the cutting of many connections, the technic (no matter how much care is exercised) is bound to give a higher weight variability, than when the removal is merely a matter of shelling an organ out of its envelope. In view of this fact the brain-spinal cord difference in variability gives no basis for an inter-organ biological interpretation.

In a preceding paragraph mention was made of "reduced variability." This is a statistical value purporting to show the variability of a variable freed from influences assumed to be exerted by the other variables being studied. I have calculated these values for the body weight, body length, brain weight and spinal cord weight, and have arranged them in the order of decreasing variability in Table IV. as a matter of record. While in every case the variability is less than that which obtains when all factors of influence are in play, the relative position of any variable in the general scheme is unchanged.

TABLE IV.

THE REDUCED VARIABILITY OF THE SEVERAL VARIABLES DISCUSSED IN THIS STUDY.

Structure.	Male.	Female.
Body Weight	11.41	9.86
Spinal Cord	5.28	5.06
Brain	4.35	4.08
Body Length	3.65	2.93

In the earlier study (1) on the weight inter-relationships of the glands of internal secretion a similar computation was made of the reduced variability of that particular array. It was found that the thyroid has the highest variability of all the organs so far studied, and that the thymus comes next. Both of these organs are much more variable than the body as a whole. The adrenals, gonads, hypophysis, and pancreas give lower figures, but in all cases values considerably higher than those for brain or spinal cord.

This generally lesser variability of the central nervous system is a demonstration that it is much more metabolically stable than are the other organs of the body so far studied. The interpretation is based on the difference in chemical make-up already discussed, in which differences in type metabolism participate. A more extended discussion of the problem is reserved for a later paper.

Summary and Conclusions.

The brain and spinal cord of the albino rat show a high degree of positive weight correlation, free from the influences assumed to be exerted by the other variables studied (body weight, body length). The value for the male is 0.567, and for the female 0.570.

The weight variability of the brain and spinal cord is less than that of any of the other organs so far studied. It is greater than that of the body in length.

The analysis and interpretation of these and other relations are given in detail in the text.

The computations upon which this study is based were made by Miss Mildred Wilson.

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THE NUTRITION OF THE OVUM OF *HYDRA VIRIDIS*.

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The incipient ovary of *Hydra viridis* is represented by a mass of proliferated interstitial cells. At a very early stage in the development of the ovary some of its cells become much larger than the others. There seems to be a struggle on between these cells; for, as development of the ovary proceeds, but one of the larger ones remains whole, and the smaller ones perish. The cells, that are directly involved in this struggle, are enclosed in a thin wall of slightly chromatic, modified epithelio-muscular cells. Kleinenberg (72), Brauer (91), Downing (08) and Tannreuther (08) agree in the statement that one of the enlarged interstitial cells gets the ascendency over the others and grows at their expense. If there be in the incipient ovary more than one greatly enlarged interstitial cell, these may fuse to form the oögonium as over against the cells of what Tannreuther (08) designates "the cells of the peripheral region which contribute to the formation of the yolk," p. 274. These peripheral cells are not taken into the growing oögonium's cytoplasm bodily as Brauer (91) described. They disintegrate at the periphery of the oögonium and are then resorbed. The relation of these disintegrating cells to the cytoplasm of the final oögonium are shown in Fig. 1. Figs. 3 and 4 show phases of disintegration in these neighboring enlarged interstitial cells. As their cytoplasm breaks down, the nuclei display disintegration features. Eventually the entire cell disintegrates. The substance of these disintegrated cells is absorbed by the oögonium, as Kleinenberg (72) described. The material thus obtained results in the oögonium growing greatly to become a conspicuously large amœboid cell (Text-figure 2). This cell, by means of radiating stout pseudopods, spreads out over one third or more of the mesoglea's outer surface. This amœboid gamete was first figured by Fewkes and Mark in 1884. This pseudopodial cell has now attained its maximum size and is, therefore,

the primary oöcyte. The feeding of the oögonium, of the final oögonial generation, upon the neighboring, enlarged interstitial cells represents the first phase of the nutrition of the ovum of *Hydra viridis*. Nutrition, in this phase, is referred to the growth of the final oögonium into the primary oöcyte.

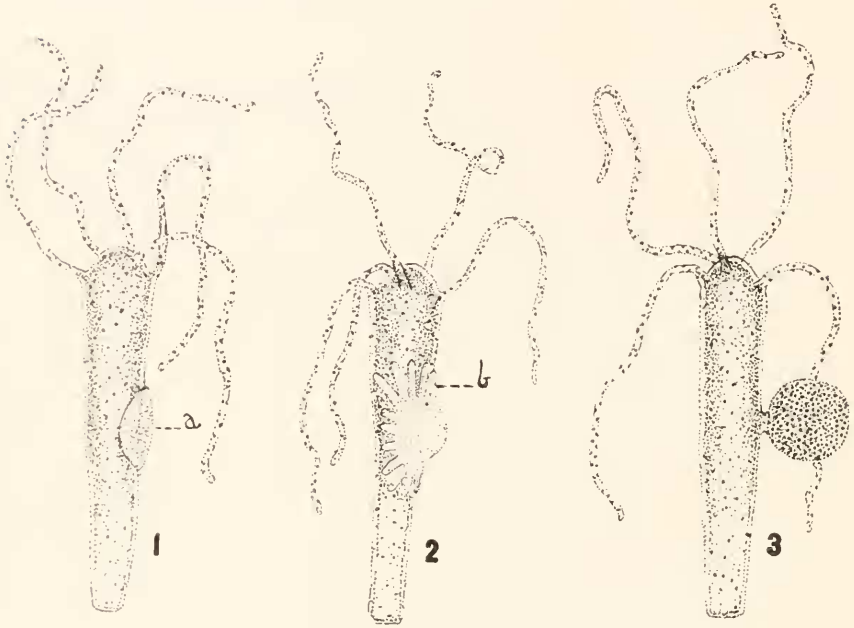

FIG. 1. Oögonium before pseudopodia are thrown off. *a* shows plane through which section, shown in figure 1 of plate, was taken.

FIG. 2. Oöcyte with maximum number of pseudopodia. *b* shows plane through which section, shown in figure 5 of plate, was taken.

FIG. 3. Advanced primary oöcyte with a full complement of deutoplasm.

Previous investigators have failed to recognize that there are two phases in the nutrition of *Hydra's* ovum. Perhaps failure, on their part, to recognize the dual nature of the nutrition of the ovum of *Hydra* arises out of the resemblance of the disintegrating interstitial nuclei to deutoplasmic granules (Fig. 3). Brauer (91) held that the nuclei of the cells, that were being ingested by the egg, became the yolk granules or his "Pseudozellen." Downing (08) says the "interstitial cells adjacent to the egg in the fairly mature ovary have their walls in contact with the egg resorbed and the content of the cell becomes part of the egg (Nusbaum).

The greatly enlarged nuclei, gorged with lecithin, also become yolk granules or "Pseudozellen," p. 66. Tannreuther (08) also looks upon the yolk granules as arising from the nuclei of the interstitial cells. He says: "When the pseudopodia are completely formed, the nuclei of the interstitial cells forming the ovary are taken up by the amœboid egg and become changed into the yolk or pseudo-cells of the egg. . . . After the yolk or pseudo-cells are formed they divide amitotically" (p. 264).

We have been unable to bring the details of *Hydra viridis* ovary in line with the above interpretations and facts.

To begin with, up until the time that the growing, amœboid oögonium has made maximum contact with the mesoglea—thus placing it in relation to the endoderm—there has been no yolk formation. In the meantime, however, many of the attending, peripherally disposed cells have disintegrated and have been resorbed. Sometimes this disintegration and resorption has gone so far that, before the oögonium has well advanced in growth, the attending cells lie only at its margin (Fig. 1). In a typical example, by the time the oögonium has reached its maximum growth, there is but a thin tissue of highly modified epidermis covering its general surface (Fig. 5). In such example, there may yet be disintegrating cells at the tips of the pseudopods or even beyond them. Thus it is to be emphasized that many of the enlarged interstitial cells have disintegrated (perhaps most of them) and have been resorbed during the oögonium's growth. And yet, up until maximum surface contact with the mesoglea has been established, no yolk formation has resulted. It should be further indicated that we have found no enlarged interstitial cells to be taken up bodily by the amœboid egg-cell. These two features of the nutrition of ovum of *Hydra* stand in contrast with what Tannreuther (08) describes. He says: "When the egg has reached its growth, it is amœboid in form with the nucleus near the center. The egg at this stage of development contains no yolk, . . . but when pseudopodia are completely formed, the nuclei of the interstitial cells forming the ovary, are taken up by the amœboid egg and become changed into yolk or pseudo-cells of the egg. Fig. 6 represents a cross-section of several pseudopodia into which the nuclei of the interstitial cells of the ovary

are passing" (p. 263–264). The contrast between what Tannreuther herein describes and our observations appears in two ways. In the first place, if yolk formation depends upon the disappearance of the interstitial cells of the ovary, then yolk should appear when these cells disappear. They are clearly seen to disappear throughout the growth of the oögonium and yet until the latter has reached its full growth no yolk has appeared. In the second place, we find that no interstitial cells have been bodily taken up or ingested as shown by Tannreuther in his Fig. 6. His Fig. 6, however, is not, in itself, convincing; for he shows the so-called nuclei leaving only two interstitial cells. Moreover, the cells from which these nuclei are migrating show no marked cytoplasmic change. Likewise, his written observations are not convincing with reference to the manner in which yolk arises. He makes the significant statement that "The pseudopodia do not grow out between the cells of the ovary, but rather between the ovary as a whole and the mesoglea" (p. 263). If, now, the pseudopodia were sent out with reference to yolk formation, dependent upon the interstitial cells of the ovary, they would "grow out between the cells of the ovary" and not "between the ovary as a whole and the mesoglea."

There is no meaning in the extensive application of the primary oöcyte's pseudopodia to the mesoglea, if the yolk granules are derived from the interstitial cells. On the other hand, we see in this spreading out of the primary oöcyte over the mesoglea a method of making maximum contact with a source of food material upon which to draw for the elaboration of deutoplasm.

In *Hydra*, the endoderm is the source of food supply. Kepner and Hopkins (24) observed that, as a diploblastic animal, *Hydra* cannot transport widely material absorbed by the endoderm. For example, chloretone injected into the enteric cavity of *Hydra* effects only the adjacent ectoderm of the body proper. The sphincters at the bases of the tentacles prevented the injected chloretone entering the latter and the compression of the walls of the peristome prevented chloretone entering its lumen. It was thus of interest to observe that the tentacles and peristome received none of the chloretone that had been absorbed by the general endoderm, for they became unusually active in contrast

to the quieted body proper. Just as chloretone could not be sent to the closed tentacles, so it appears the endoderm of *Hydra* cannot send food-material along a narrow channel to its oögonium. The growing oögonium must, therefore, come to the endoderm. As a result of this imposition, by the time the final oögonium has become, through growth, a primary oöcyte, an extended relation between the latter and the endoderm has been established. This relation established marks the inception of the second phase of the nutrition of the ovum of *Hydra viridis*. At the beginning of this second nutritional phase, there are no deutoplasmic inclusions within the cytoplasm. Soon, however, yolk is formed within the cytoplasm of the oöcyte (Fig. 5). This deutoplasm is elaborated by the oöcyte out of material taken over in solution from the endoderm and assimilated by the female gamete. Thus the deutoplasm may be looked upon as material elaborated by the oöcyte. The deutoplasmic granules are not to be considered the lineal descendants of original nuclei of neighboring interstitial cells that have come to be more and more numerous through amitosis. This position seems logical when we bear in mind the fact that, though many interstitial cells have disintegrated (perhaps most of them) and have been resorbed during the egg's growth, yet, up until maximum surface exposure to the endoderm has been made, no yolk-formation has resulted. Our interpretation is further strengthened by the observation that so long as yolk is making its appearance within the primary oöcyte a maximum surface relation to the endoderm is maintained; but when the maximum amount of yolk has been formed the egg retreats from the endoderm as Tannreuther (08) indicates: "After the amœboid egg becomes filled with yolk, the pseudopodia are drawn in and the egg becomes nearly spherical" (p. 264), (Text-figure, 3). The second phase of the nutrition of *Hydra viridis*, therefore, ends with the retreat of the primary oöcyte from the mesoglea after it has become filled with deutoplasm. No deutoplasm is formed thereafter. This phase of nutrition is referred to the development of the zygote.

Summary.

The nutrition of *Hydra viridis* is a dual process, there being two phases.

The first phase has reference to the nutrition of an oögonium of the final generation. This oögonium is nourished through the disintegration and resorption of adjacent interstitial cells. Through the nourishment, thus obtained, the final oögonium grows into a large pseudopodial cell, the primary oöcyte. The first nutritional phase is referred to the growth of the final oögonium into a primary oöcyte. It does not involve yolk-formation.

The second phase of nutrition begins with the primary oöcyte lying, as a pseudopodial cell, in extended relation to the endoderm. Yolk is elaborated by the oöcyte from material handed over by the endoderm and the protoplasm of interstitial cells is not involved. The second nutritional phase is referred to the development of the zygote.

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EXPLANATION OF PLATE.

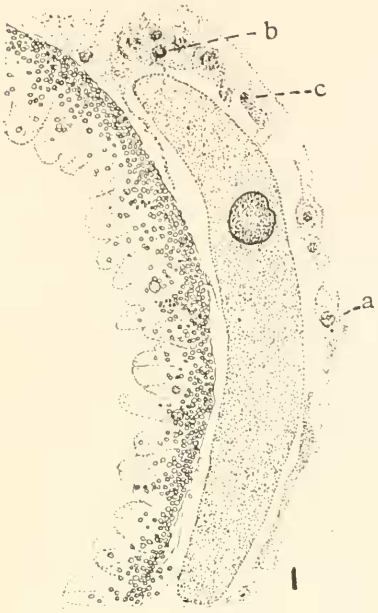
FIG. 1. Part of a section taken through plane indicated at *a* in text-figure. A group of disintegrating interstitial cells is shown at *b*, *c*. *a* shows that already, in this particular example, the superimposed ectoderm was but a single layer thick. × 250.

FIG. 2. Cell *a* of Fig. 1, magnified to indicate the character of cell that forms ovarian wall. × 1,250.

FIG. 3. Cell *b* in Fig. 1. Shows a disintegrating interstitial cell in which the nucleus resembles a deutoplasmic granule. × 1,250.

FIG. 4. Cell *c* in Fig. 1. An attending interstitial cell in a more advanced phase of disintegration than cell shown in Fig. 3. The nucleus no longer resembles a deutoplasmic granule. × 1,250.

FIG. 5. Section taken through plane indicated at *b* in text-figure. Shows eleven pseudopodia closely applied to mesoglea. Yolk-formation has begun; it is not, however, completed though all enlarged interstitial cells have disappeared. × 250.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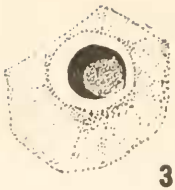
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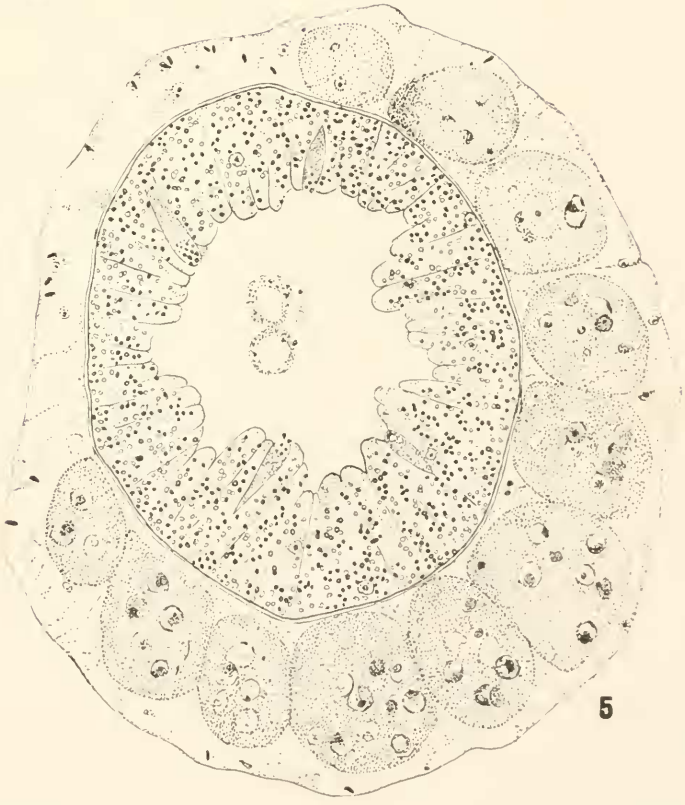
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